(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 August 2002 (29.08.2002)

PCT

(10) International Publication Number
WO 02/065988 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number: PCT/US02/04781

(22) International Filing Date: 19 February 2002 (19.02.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/270,009 20 February 2001 (20.02.2001) US

(71) Applicant: **ENZON, INC.** [US/US]; 20 Kingsbridge Road, Piscataway, NJ 08854 (US).

(72) Inventors: **CHOE, Yun, Hwang**; 9 Greenbrier Road, Green Brook, NJ 08812 (US). **GREENWALD, Richard, B.**; 113 Hickory Road, Somerset, NJ 08873 (US).

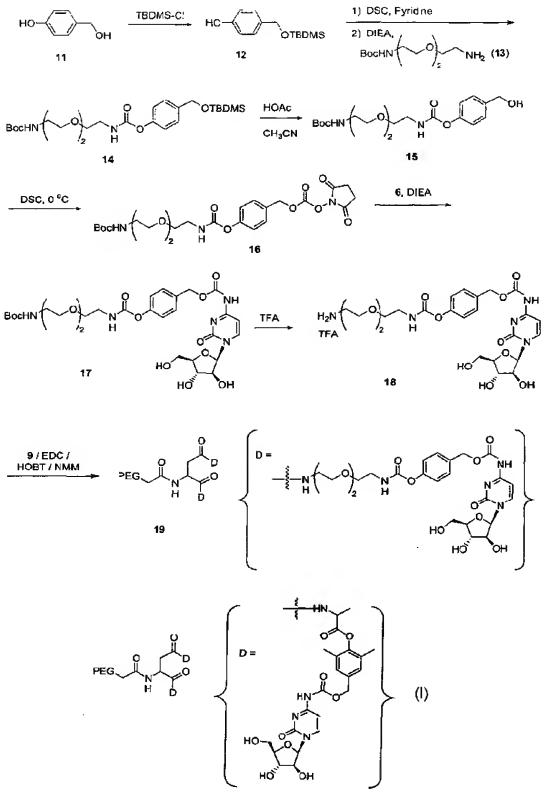
(74) Agent: **MERCANTI, Michael, N.**; Roberts & Mercanti, L.L.P., EDC II, 105 Lock Street, Suite 203, Newark, NJ 07103 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: TERMINALLY-BRANCHED POLYMERIC LINKERS AND POLYMERIC CONJUGATES CONTAINING THE SAME



(57) Abstract: Terminally-branched polymeric prodrug platforms capable of high degrees of loading are disclosed. In preferred aspects of the invention, the prodrug platform releases multiple parent compounds after each branch holding the active agent undergoes a benzyl elimination reaction. Methods of preparing the prodrugs and using the same in the treatment of mammals are also disclosed. In one preferred aspect, polymeric conjugates such as (I) are provided.

WO 02/065988 A2

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TERMINALLY-BRANCHED POLYMERIC LINKERS AND POLYMERIC CONJUGATES CONTAINING THE SAME

TECHNICAL FIELD

The present invention relates to new types of terminally-activated polymeric materials which are useful in forming long-acting conjugates of bioactive materials. In particular, the invention relates to polymeric-based conjugates having increased therapeutic payloads and methods of preparing the same.

BACKGROUND OF THE INVENTION

Over the years, several methods of administering biologically-effective materials to mammals have been proposed. Many medicinal agents are available as water-soluble salts and can be included in pharmaceutical formulations relatively easily. Problems arise when the desired medicinal agent is either insoluble in aqueous fluids or is rapidly degraded in vivo. Alkaloids are often especially difficult to solubilize.

One way to solubilize medicinal agents is to include them as part of a soluble prodrug. Prodrugs include chemical derivatives of a biologically-active parent compound which, upon administration, eventually liberate the parent compound in vivo. Prodrugs allow the artisan to modify the onset and/or duration of action of an agent in vivo and can modify the transportation, distribution or solubility of a drug in the body. Furthermore, prodrug formulations often reduce the toxicity and/or otherwise overcome difficulties encountered when administering pharmaceutical preparations. Typical examples of prodrugs include organic phosphates or esters of alcohols or thioalcohols. See Remington's Pharmaceutical Sciences, 16th Ed., A. Osol, Ed. (1980), the disclosure of which is incorporated by reference herein.

Prodrugs are often biologically inert or substantially inactive forms of the parent or active compound. The rate of release of the active drug, i.e. the rate of hydrolysis, is

influenced by several factors but especially by the type of bond joining the parent drug to the modifier. Care must be taken to avoid preparing prodrugs which are eliminated through the kidney or reticular endothelial system, etc. before a sufficient amount of hydrolysis of the parent compound occurs.

Incorporating a polymer as part of a prodrug system has been suggested to increase the circulating life of a drug. However, it has been determined that when only one or two polymers of less than about 10,000 daltons each are conjugated to certain biologically active substances such as alkaloid compounds, the resulting conjugates are rapidly eliminated in vivo, especially if a somewhat hydrolysis-resistant linkage is used. In fact, such conjugates are so rapidly cleared from the body that even if a hydrolysis-prone ester linkage is used, not enough of the parent molecule is regenerated in vivo to be therapeutic.

Camptothecin and related biologically active analogs are often poorly water soluble and are examples of substances which would benefit from PEG prodrug technology. A brief overview of some previous work in the field is presented below.

Ohya, et al., J. Bioactive and Compatible Polymers Vol. 10 Jan., 1995, 51-66, disclose doxorubicin-PEG conjugates which are prepared by linking the two substituents via various linkages including esters. The molecular weight of the PEG used, however, is only about 5,000 at most. Thus, the in vivo benefits are not fully realized because the conjugates are substantially excreted prior to sufficient linkage hydrolysis.

U.S. Patent No. 4,943,579 discloses certain simple 20(S)-camptothecin amino acid esters in their salt forms as water soluble prodrugs. The reference does not, however, disclose using an amino acid as part of a linkage which would attach the alkaloid to a relatively high molecular weight polymer in order to form a prodrug. As evidenced by the data provided in Table 2 of the '579 patent, hydrolysis is rapid. Consequently, at physiologic pH, the insoluble base is rapidly generated after injection, binds to proteins and is quickly eliminated from the body before a therapeutic effect can be achieved. A related effort was directed to developing a water-soluble camptothecin sodium salt. Unfortunately, the water-soluble sodium salt of camptothecin remained too toxic for clinical application (Gottlieb et al,. 1970 Cancer Chemother. Rep. 54, 461; Moertel et al,. 1972 ibid, 56, 95; Gottlieb et al., 1972 ibid, 56, 103).

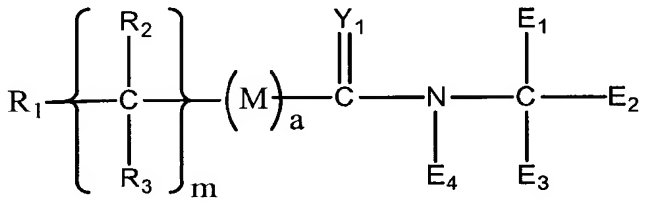
Commonly-assigned PCT publication WO96/23794 describes bis-conjugates in which one equivalent of the hydroxyl-containing drug is attached to each terminal of the polymer. In spite of this advance, techniques which would further increase the payload of the polymer have been sought.

Thus, there continues to be a need to provide additional technologies for forming prodrugs of therapeutic moieties such as camptothecin and related analogs. The present invention addresses this need.

SUMMARY OF THE INVENTION

In one aspect of the invention, compounds of Formula (I) are provided:

(I)



wherein:

R_1 is a polymeric residue;

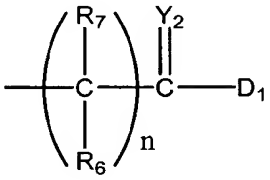
Y_1 is O, S or NR_4;

M is O, S or NR_5;

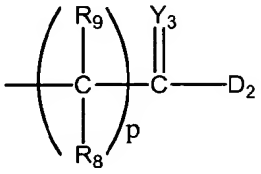
(m) is zero or a positive integer, preferably 1 or 2;

(a) is zero or one;

E_1 is



E_{2-4} are independently H, E_1 or



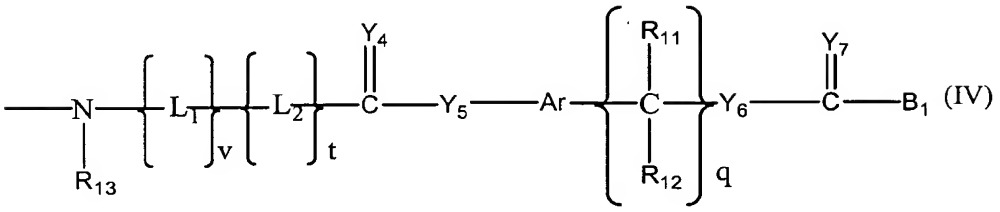
(n) and (p) are independently 0 or a positive integer;

Y_{2-3} are independently O, S or NR_{10};

R_{2-10} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} hetero-

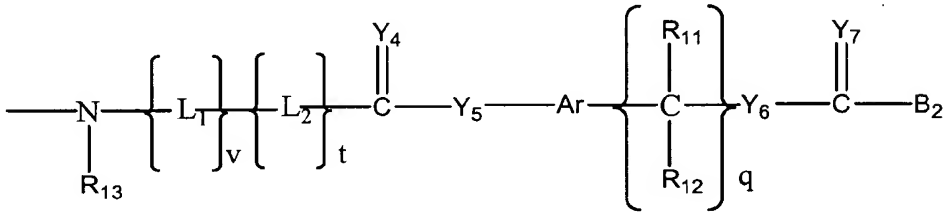
alkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

D_1 and D_2 are independently OH,



(IV)

(V)



or additional terminal branching groups described below.

Within formulae (IV) and (V), the variables (v) and (t) are independently 0 or a positive integer up to about 6, and preferably about 1;

(q) is zero or a positive integer, preferably one;

L_1 and L_2 are independently selected bifunctional linkers;

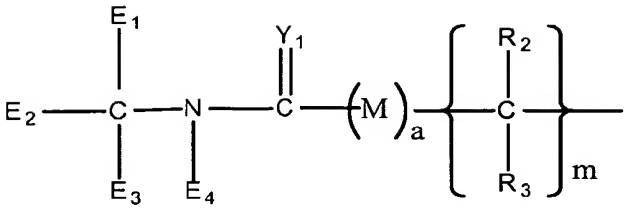
Y_{4-7} are independently selected from the group consisting of O, S and NR_{14};

R_{11-14} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroakoxy;

Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group; and

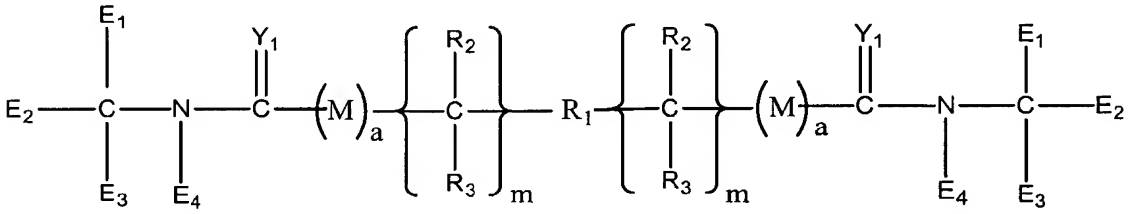
B_1 and B_2 are independently selected from the group consisting of leaving groups, OH, residues of hydroxyl-containing moieties or amine-containing moieties.

In one particularly preferred aspect of the invention, the polymeric residue is also substituted or capped on the distal portion with a moiety of formula (II) below:



(II)

where all variables are as previously defined. Bifunctional compounds are thus formed when the polymeric residue (R_1) includes both an alpha and an omega terminal linking group so that two, four or more equivalents of a biologically active agent, drug or protein, designated herein as B_1 or B_2 can be delivered. An example of such a bifunctional polymer transport form is illustrated below as formula (III):



(III)

wherein all variables are as described above.

For purposes of the present invention, the term "residue" shall be understood to mean that portion of a biologically active compound which remains after the biologically active compound has undergone a substitution reaction in which the prodrug carrier portion has been attached.

For purposes of the present invention, the term "alkyl" shall be understood to include straight, branched, substituted, e.g. halo-, alkoxy-, and nitro-,C_{1-12} alkyls, C_{3-8} cycloalkyls or substituted cycloalkyls, etc.

For purposes of the present invention, the term "substituted" shall be understood to include adding or replacing one or more atoms contained within a functional group or compound with one or more different atoms.

For purposes of the present invention, substituted alkyls include carboxyalkyls, aminoalkyls, dialkylaminos, hydroxyalkyls and mercaptoalkyls; substituted cycloalkyls include moieties such as 4-chlorocyclohexyl; aryls include moieties such as napthyl; substituted aryls include moieties such as 3-bromo-phenyl; aralkyls include moieties such

as toluyl; heteroalkyls include moieties such as ethylthiophene; substituted heteroalkyls include moieties such as 3-methoxy-thiophene; alkoxy includes moieties such as methoxy; and phenoxy includes moieties such as 3-nitrophenoxy. Halo- shall be understood to include fluoro, chloro, iodo and bromo.

The term "sufficient amounts" for purposes of the present invention shall mean an amount which achieves a therapeutic effect as such effect is understood by those of ordinary skill in the art.

One of the chief advantages of the compounds of the present invention is that the prodrugs have a higher payload per unit of polymer than previous techniques. It is generally preferred that the polymeric first releases the benzyl elimination (BE) based prodrug intermediate by hydrolysis and then the resultant intermediate or "second prodrug" moiety undergoes a 1,4- or 1,6-aryl (e.g., benzyl) elimination reaction to regenerate, for example, a moiety which is either a biologically active compound or a composition comprising a further prodrug. The high payload polymeric conjugates of the present invention are thus unique delivery systems which can contain up to four or a greater number of molecules of a drug.

Methods of making and using the compounds and conjugates described herein are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

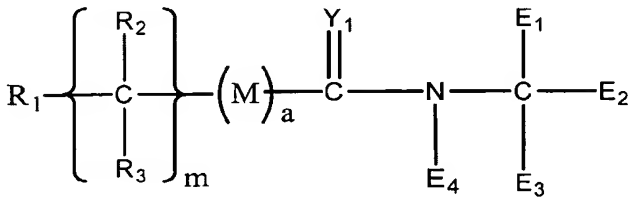
Figures 1 -5 schematically illustrate methods of forming compounds of the present invention which are described in the Examples.

DETAILED DESCRIPTION OF THE INVENTION

A. FORMULA (I)

In one preferred embodiment of the invention, there are provided compounds of the formula:

(I)

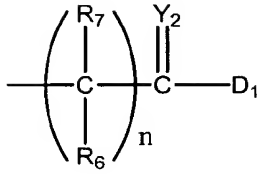


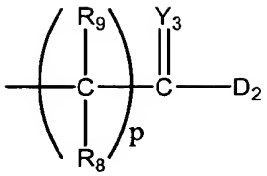
wherein:

R_1 is a polymeric residue;

Y_1 is O, S or NR_4;

M is O, S or NR_5;

E_1 is 

E_{2-4} are independently H, E_1 or 

(a) is zero or one;

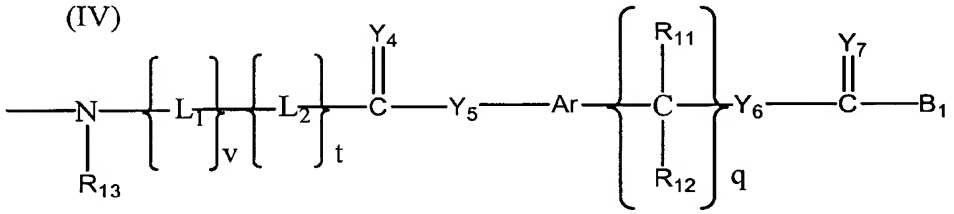
(m) is zero or a positive integer;

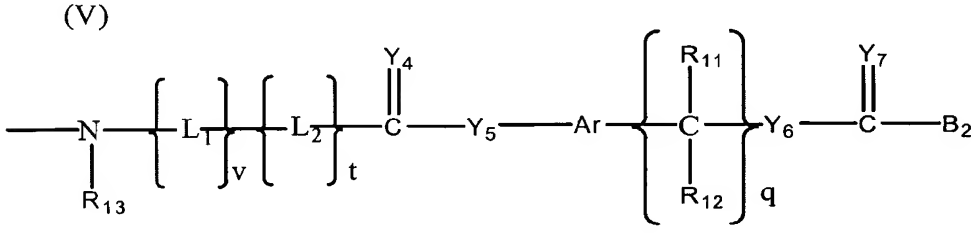
(n) and (p) are independently 0 or a positive integer;

Y_{2-3} are independently O, S or NR_{10};

R_{2-10} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

D_1 and D_2 are independently OH,

(IV) 

(V) 

wherein (v) and (t) are independently 0 or a positive integer up to about 6, and preferably about 1;

(q) is zero or a positive integer, preferably one;

L_1 and L_2 are independently selected bifunctional linkers;

Y_{4-7} are independently selected from the group consisting of O, S and NR_{14};

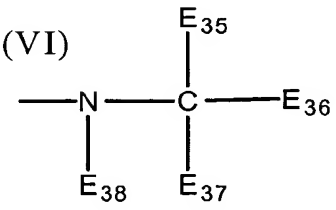
R_{11-14} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroakoxy;

Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group;

B_1 and B_2 are independently selected from the group consisting of leaving groups, OH, residues of hydroxyl-containing moieties or amine-containing moieties.

In another preferred embodiment, D_1 and D_2 are independently selected terminal branching groups of formula (VI)

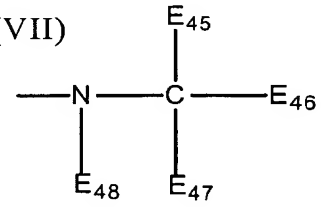
(VI)



wherein E_{35-38} are selected from the same group which defines E_{1-4} above, except that within the definition, D_1 and D_2 are changed to D'_1 and D'_2 which are defined below.

Within this embodiment, D'_1 and D'_2 can be independently OH, a moiety of formula (IV) or (V), or

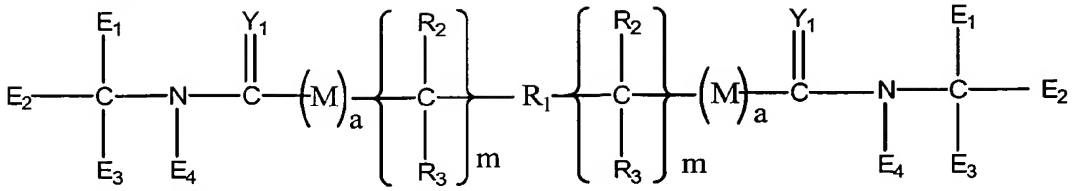
(VII)



wherein E_{45-48} are selected from the same group which defines E_{1-4}, except that within the definition D_1 and D_2 are changed to D''_1 and D''_2 and D''_1 and D''_2 are independently OH, formula (IV) or formula (V). As can be appreciated from the above, when the terminal branching is taken to its fullest extent with a bifunctional polymer R_1, up to sixteen (16) equivalents of drug can be loaded onto the polymeric platform.

In those aspects of this embodiment where bis-substituted polymeric residues are desired, some preferred polymeric transport systems of the invention are shown below as

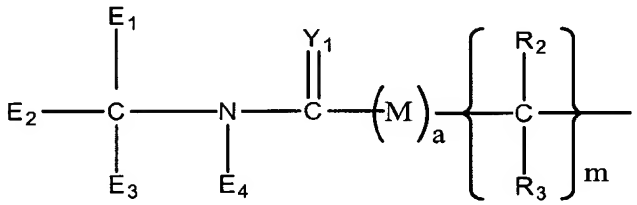
formula (III):



wherein all variables are as previously described.

The multi-loading polymer transport system of the present invention is based in large part on the polymeric residue designated herein as R_1. Optionally, R_1 includes a capping group A. The polymer capping group A includes, for example, moieties such as hydrogen, CO_2H, C_{1-6} alkyl moieties, and compounds of formula (II) shown below, which forms a bis-system:

(II)



wherein all variables are as previously described. It will be understood and appreciated that the multiple terminal branching described above applies equally in the bis-systems as well.

With regard to the other variables which comprise the formulae of the present invention, the following are preferred:

Y_{1-4} and Y_7 are each oxygen;

R_{2-14} are each preferably hydrogen or lower alkyl, e.g. C_{1-6};

(m) is 1 or 2;

(n) and (p) are each either zero or an integer from 1-4;

(v) is zero or 1;

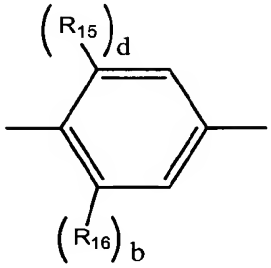
(t) is 1;

L_1 is $-(CH_2CH_2O)_2-$; and

L_2 is one of $-CH_2-$, $-CH(CH_3)-$, $-CH_2C(O)NHCH(CH_3)-$, $-(CH_2)_2-$, $-(CH_2)_2-NH-$, $-CH_2C(O)NHCH_2-$, $-(CH_2)_2-NH-C(O)(CH_2)_2NH-$ or $-CH_2C(O)NHCH(CH_2CH(CH_3)_2)-$.

B. DESCRIPTION OF THE Ar MOIETY

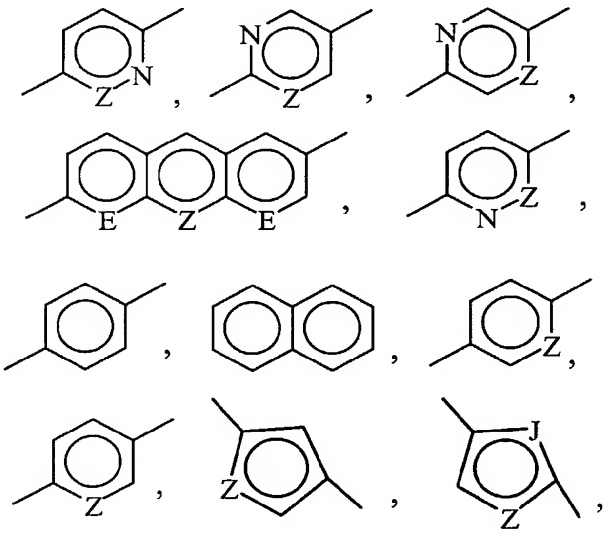
Referring to Formula (I), it can be seen that the Ar is a moiety, which when included in Formula (I), forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group. A key feature is that the Ar moiety is aromatic in nature. Generally, to be aromatic, the π electrons must be shared within a "cloud" both above and below the plane of a cyclic molecule. Furthermore, the number of π electrons must satisfy the Hückle rule (4n+2). Those of ordinary skill will realize that a myriad of moieties will satisfy the aromatic requirement of the moiety and thus are suitable for use herein. Some particularly preferred aromatic groups include:

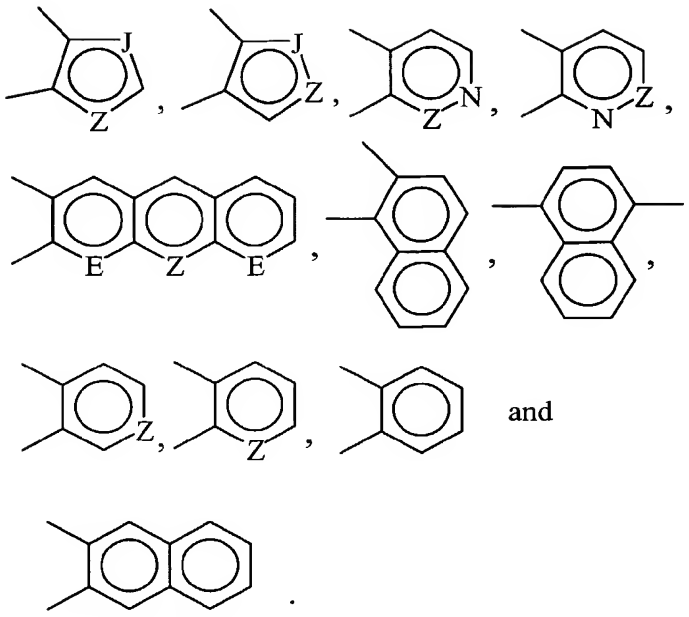


where $R_{15\text{-}16}$ are individually selected from the same group which defines R_2 and (b) and (d) are independently zero or one.

Other preferred aromatic hydrocarbon moieties include, without limitation:

:





In the above-listed aromatic moieties, J is O, S, or N-R_{17}, E and Z are independently C-R_{18} or N-R_{19}; and R_{17-19} are independently selected from the same group as that which defines R_2 in Formula (I) e.g., hydrogen, C_{1-6} alkyls, etc. Isomers of the five and six-membered rings are also contemplated as well as benzo- and dibenzo- systems and their related congeners are also contemplated. It will also be appreciated by the artisan of ordinary skill that the aromatic rings can optionally be substituted with hetero-atoms such as O, S, NR_{17}, etc. so long as Hückel's rule is obeyed. Furthermore, the aromatic or heterocyclic structures may optionally be substituted with halogen(s) and/or side chains as those terms are commonly understood in the art. However, all structures suitable for Ar moieties of the present invention are capable of allowing the Y_5 and $C(R_{11})(R_{12})$ moieties to be in a *para* or an *ortho* arrangement with the same plane.

C. DRUG GENERATION VIA HYDROLYSIS OF THE PRODRUG

The prodrug compounds of the present invention are designed so that the $t_{1/2}$ of hydrolysis is < $t_{1/2}$ elimination in plasma.

The linkages included in the compounds have hydrolysis rates in the plasma of the mammal being treated which is short enough to allow sufficient amounts of the parent compounds, i.e. the amino- or hydroxyl-containing bioactive compound, to be released prior to elimination. Some preferred compounds of the present invention have a $t_{1/2}$ for

hydrolysis in plasma ranging from about 5 minutes to about 12 hours. Preferably, the compositions have a plasma $t_{1/2}$ hydrolysis ranging from about 0.5 to about 8 hours and most preferably from about 1 to about 6 hours.

D. SUBSTANTIALLY NON-ANTIGENIC POLYMERS

As stated above, R_1 is a water soluble polymeric residue which is preferably substantially non-antigenic such as a polyalkylene oxide (PAO) or polyethylene glycol (PEG). In preferred aspects of the invention, R_1 further includes the previously mentioned capping group, designated herein as A, which allows a bifunctional or bis-polymer system to be formed.

As an example, the PEG residue portion of the inventive compositions can be selected from the following non-limiting list:

$-C(=Y_8)-(CH_2)_f-O-(CH_2CH_2O)_x-A$,

$-C(=Y_8)-Y_9-(CH_2)_f-O-(CH_2CH_2O)_x-A$,

$-C(=Y_8)-NR_{20}-(CH_2)_f-O-(CH_2CH_2O)_x-A$,

$-(CR_{21}R_{22})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-A$,

$-NR_{20}-(CH_2)_f-O-(CH_2CH_2O)_x-A$,

$-C(=Y_8)-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-C(=Y_8)-$,

$-C(=Y_8)-Y_9-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-Y_9-C(=Y_8)-$,

$-C(=Y_8)-NR_{20}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{20}-C(=Y_8)-$,

$-(CR_{21}R_{22})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-O-(CR_{21}R_{22})_e-$, and

$-NR_{20}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{20}-$

wherein:

Y_8 and Y_9 are independently O, S or NR_{20};

x is the degree of polymerization;

R_{20}, R_{21} and R_{22} are independently selected from among H, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

e and *f* are independently zero, one or two; and

A is a capping group.

The degree of polymerization for the polymer (x) can be from about 10 to about

2,300. This represents the number of repeating units in the polymer chain and is dependent on the molecular weight of the polymer. The (A) moiety is a capping group as defined herein, i.e. a group which is found on the terminal of the polymer and, in some aspects, can be selected from any of H, NH_2, OH, CO_2H, C_{1-6} alkyls or other PEG terminal activating groups, as such groups are understood by those of ordinary skill.

Also useful are polypropylene glycols, branched PEG derivatives such as those described in commonly-assigned U.S. Patent No. 5,643,575, "star-PEG's" and multi-armed PEG's such as those described in Shearwater Polymers, Inc. catalog "Polyethylene Glycol Derivatives 1997-1998". The disclosure of each of the foregoing is incorporated herein by reference. It will be understood that the water-soluble polymer can be functionalized for attachment to the bifunctional linkage groups if required without undue experimentation.

In a further embodiment R_1 is optionally selected from among one or more of dextran, polyvinyl alcohols, carbohydrate-based polymers, hydroxypropylmethacryl-amide, polyalkylene oxides, and/or copolymers thereof. *See* also commonly-assigned U.S. Patent No, 6,153,655, the contents of which are incorporated herein by reference.

In many aspects of the present invention, bis-activated polyethylene glycols are preferred when di-or more substituted polymer conjugates are desired. Alternatively, polyethylene glycols (PEG's), mono-activated, C_{1-4} alkyl-terminated polyalkylene oxides (PAO's) such as mono-methyl-terminated polyethylene glycols (mPEG's) are preferred when mono-substituted polymers are desired.

In order to provide the desired hydrolyzable linkage, mono- or di-acid activated polymers such as PEG acids or PEG diacids can be used as well as mono- or di-PEG amines and mono- or di-PEG diols. Suitable PAO acids can be synthesized by first converting mPEG-OH to an ethyl ester followed by saponification. See also Gehrhardt, H., et al. Polymer Bulletin 18: 487 (1987) and Veronese, F.M., et al., J. Controlled Release 10; 145 (1989). Alternatively, the PAO-acid can be synthesized by converting mPEG-OH into a *t*-butyl ester followed by acid cleavage. See, for example, commonly assigned U.S. Patent No. 5,605,976. The disclosures of each of the foregoing are incorporated by reference herein.

Although PAO's and PEG's can vary substantially in average molecular weight, the polymer portion of the prodrug is at least about 20,000 weight average in most aspects

of the invention. Preferably, R_1 has a weight average molecular weight of from about 20,000 to about 100,000 and more preferably from about 25,000 to about 60,000. The average molecular weight of the polymer selected for inclusion in the prodrug must be sufficient so as to provide sufficient circulation of the prodrug before hydrolysis of the linker.

The polymeric substances included herein are preferably water-soluble at room temperature. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymers is maintained.

As an alternative to PAO-based polymers, effectively non-antigenic materials such as dextran, polyvinyl alcohols, carbohydrate-based polymers, hydroxypropylmethacrylamide (HPMA), and copolymers thereof etc. and the like can be used if the same type of activation is employed as described herein for PAO's such as PEG. Those of ordinary skill in the art will realize that the foregoing list is merely illustrative and that all polymeric materials having the qualities described herein are contemplated. For purposes of the present invention, "effectively non-antigenic" and "substantially non-antigenic" shall be understood to include all polymeric materials understood in the art as being substantially non-toxic and not eliciting an appreciable immune response in mammals.

It will be clear from the foregoing that other polyalkylene oxide derivatives of the foregoing, such as the polypropylene glycol acids, etc., as well as other bi- functional linking groups are also contemplated.

E. PRODRUG CANDIDATES

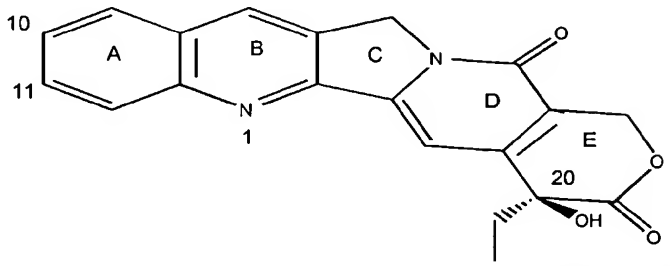
1. Residues of Hydroxyl-containing Compounds

a. Camptothecin and Related Topoisomerase I Inhibitors

Camptothecin is a water-insoluble cytotoxic alkaloid produced by *Camptotheca accuminata* trees indigenous to China and *nothapodytes foetida* trees indigenous to India. Camptothecin and related compounds and analogs are also known to be potential anticancer or antitumor agents and have been shown to exhibit these activities in vitro and in vivo. Camptothecin and related compounds are also candidates for conversion to the

prodrugs of the present invention.

Camptothecin and certain related analogues share the structure:



From this core structure, several known analogs have been prepared. For example, the A ring in either or both of the 10- and 11-positions can be substituted with an OH. The A ring can also be substituted in the 9-position with a straight or branched C_{1-30} alkyl or C_{1-17} alkoxy, optionally linked to the ring by a heteroatom i.e.- O or S. The B ring can be substituted in the 7-position with a straight or branched C_{1-30} alkyl or substituted alkyl-, C_{5-8} cycloakyl, C_{1-30} alkoxy, phenyl alkyl, etc., alkyl carbamate, alkyl carbazides, phenyl hydrazine derivatives, amino-, aminoalkyl-, aralkyl, etc. Other substitutions are possible in the C, D and E rings. See, for example, U.S. Patent Nos. 5,004,758; 4,943,579; Re 32,518, the contents of which are incorporated herein by reference. Such derivatives can be made using known synthetic techniques without undue experimentation. Preferred camptothecin derivatives for use herein include those which include a 20-OH or another OH moiety which is capable of reacting directly with activated forms of the polymer transport systems described herein or to the linking moiety intermediates, e.g. iminodiacetic acid, etc., which are then attached to a polymer such as PEG. Reference to camptothecin analogs herein has been made for purposes of illustration and not limitation.

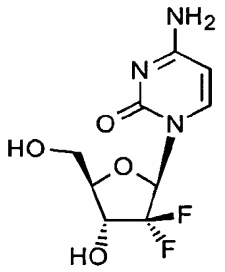
b. Taxanes and Paclitaxel Derivatives

One class of compounds included in the prodrug compositions of the present invention is taxanes. For purposes of the present invention, the term "taxane" includes all compounds within the taxane family of terpenes. Thus, taxol (paclitaxel), 3'-substituted tert-butoxy-carbonyl-amine derivatives (taxoteres) and the like as well as other analogs which are readily synthesized using standard organic techniques or are available from commercial sources such as Sigma Chemical of St. Louis, Missouri are within the scope

of the present invention. These derivatives have been found to be effective anti-cancer agents. Numerous studies indicate that the agents have activity against several malignancies. To date, their use has been severely limited by, among other things, their short supply, poor water solubility and a tendency to cause hypersensitivity. It is to be understood that other taxanes including the 7-aryl-carbamates and 7-carbazates disclosed in commonly assigned U.S. Patent Nos. 5,622,986 and 5,547,981 can also be included in the prodrugs of the present invention. The contents of the foregoing U.S. patents are incorporated herein by reference. Paclitaxel is a preferred taxane.

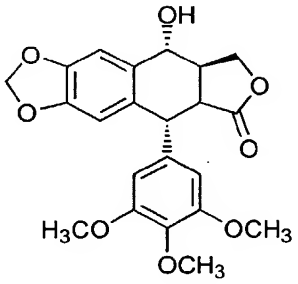
c. Additional Biologically-Active Moieties

In addition to the foregoing molecules, the prodrug formulations of the present invention can be prepared using many other compounds. For example, biologically-active compounds such as bis-PEG conjugates derived from compounds such as gemcitabine:



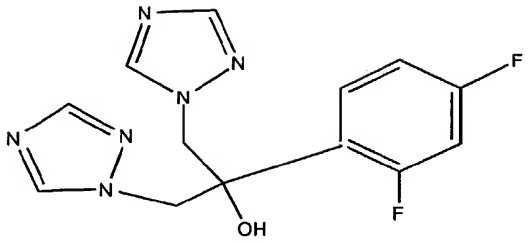
or

podophyllotoxin:

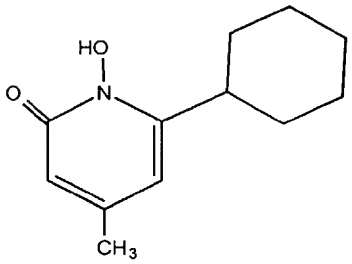


or

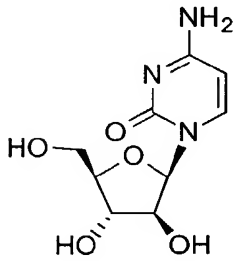
triazole-based antifungal agents such as fluconazole:



or ciclopirox:



or Ara-C:



or known derivatives thereof, including N^4 amino acid derivatives such as leu-Ara-C.

The parent compounds selected for prodrug forms need not be substantially water-insoluble, although the polymer-based prodrugs of the present invention are especially well suited for delivering such water-insoluble compounds. Other useful parent compounds include, for example, certain low molecular weight biologically active proteins, enzymes and peptides, including peptido glycans, as well as other anti-tumor agents; cardiovascular agents such as forskolin; anti-neoplastics such as combretastatin, vinblastine, doxorubicin, maytansine, etc.; anti-infectives such as vancomycin, erythromycin, etc.; anti-fungals such as nystatin, amphotericin B, triazoles, papulocandins, pneumocandins, echinocandins, polyoxins, nikkomycins, pradimicins, benanomicins, etc. see, "Antibiotics That Inhibit Fungal Cell Wall Development" Annu. Rev. Microbiol. 1994, 48:471-97, the contents of which are incorporated herein by reference; anti-anxiety agents, gastrointestinal agents, central nervous system-activating

agents, analgesics, fertility or contraceptive agents, anti-inflammatory agents, steroidal agents, anti-urecemic agents, cardiovascular agents, vasodilating agents, vasoconstricting agents and the like.

The foregoing is illustrative of the biologically active moieties which are suitable for the prodrugs of the present invention. It is to be understood that those biologically active materials not specifically mentioned but having suitable ester-forming groups, i.e. hydroxyl moieties, are also intended and are within the scope of the present invention. It is also to be understood that the prodrug conjugates of the present invention may also include minor amounts of compounds containing not only one equivalent of drug and polymer but also a moiety which does not effect bioactivity in vivo. For example, it has been found that in some instances, in spite of reacting diacids with drug molecules having a single linkage point, the reaction conditions do not provide quantitative amounts of prodrugs with two equivalents of drug per polymer. By-products of the reactants can sometimes be formed such as acyl ureas if carbodiimides are used.

2. Residues of Amine-containing Compounds

In some aspects of the invention, B_1 or B_2 is a residue of an amine-containing compound, a non-limiting list of such suitable compounds include residues of organic compounds, enzymes, proteins, polypeptides, etc. Organic compounds include, without limitation, moieties such as anthracycline compounds including daunorubicin, doxorubicin; *p*-aminoaniline mustard, melphalan, Ara-C (cytosine arabinoside) and related anti-metabolite compounds, e.g., gemcitabine, etc. Alternatively, B can be a residue of an amine-containing cardiovascular agent, anti-neoplastic, anti-infective, anti-fungal such as nystatin and amphotericin B, anti-anxiety agent, gastrointestinal agent, central nervous system-activating agent, analgesic, fertility agent, contraceptive agent, anti-inflammatory agent, steroidal agent, anti-urecemic agent, vasodilating agent, vasoconstricting agent, etc.

In a preferred aspect of the invention, the amino-containing compound is a biologically active compound that is suitable for medicinal or diagnostic use in the treatment of animals, e.g., mammals, including humans, for conditions for which such treatment is desired. The foregoing list is meant to be illustrative and not limiting for the compounds which can be modified. Those of ordinary skill will realize that other such compounds can be similarly modified without undue experimentation. It is to be

understood that those biologically active materials not specifically mentioned but having suitable amino-groups are also intended and are within the scope of the present invention.

The only limitations on the types of amino-containing molecules suitable for inclusion herein is that there is available at least one (primary or secondary) amine-containing position which can react and link with a carrier portion and that there is not substantial loss of bioactivity after the prodrug system releases and regenerates the parent compound.

It is noted that parent compounds suitable for incorporation into the prodrug compositions of the invention, may themselves be substances/compounds which are not active after hydrolytic release from the linked composition, but which will become active after undergoing a further chemical process/reaction. For example, an anticancer drug that is delivered to the bloodstream by the double prodrug transport system, may remain inactive until entering a cancer or tumor cell, whereupon it is activated by the cancer or tumor cell chemistry, e.g., by an enzymatic reaction unique to that cell.

3. Leaving Groups

In those aspects where B_1 or B_2 is a leaving group, suitable leaving groups include, without limitations, moieties such as N-hydroxybenzotriazolyl, halogen, N-hydroxyphthalimidyl, p-nitrophenoxy, imidazolyl, N-hydroxysuccinimidyl, thiazolidinyl thione, or other good leaving groups as will be apparent to those of ordinary skill. The synthesis reactions used and described herein will be understood by those of ordinary skill without undue experimentation.

For example, an acylated intermediate of formula (I) can be reacted with a reactant such as 4-nitrophenyl chloroformate, disuccinimidyl carbonate (DSC), carbonyldiimidazole, thiazolidine thione, etc. to provide the desired activated derivative.

The selective acylation of the phenolic or anilinic portion of the p-hydroxybenzyl alcohol or the p-aminobenzyl alcohol and the o-hydroxbenzyl alcohol or the o-amino-benzyl alcohol can be carried out with, for example, thiazolidine thione activated polymers, succinimidyl carbonate activated polymers, carboxylic acid activated polymers, blocked amino acid derivatives. Once in place, the "activated" form of the PEG prodrug (or blocked prodrug) is ready for conjugation with an amine- or hydroxyl-containing compound.

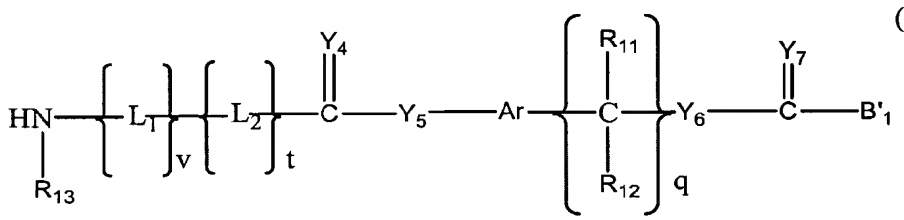
F. **SYNTHESIS OF THE POLYMERIC PRODRUG TRANSPORT SYSTEM**

Synthesis of representative polymer prodrugs is set forth in the Examples. Generally, however, in one preferred method of preparing the prodrug transport systems, the polymer residue is first attached to the branching groups. Separately, the biologically active moiety or drug, e.g. Drug-OH or Drug-NH_2 (B_1 or B_2 of formula I) is attached to the BE component which may also include a bifunctional spacer thereon at point of attachment to the polymer. Next, the polymeric residue containing the terminal branches is reacted with the drug-BE portion under conditions sufficient to form the final product.

Attachment of the bifunctional spacer containing the BE- Drug component to the polymer portion is preferably carried out in the presence of a coupling agent. A non-limiting list of suitable coupling agents include 1,3-diisopropylcarbodiimide (DIPC), any suitable dialkyl carbodiimides, 2-halo-1-alkyl-pyridinium halides, (Mukaiyama reagents), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (EDC), propane phosphonic acid cyclic anhydride (PPACA) and phenyl dichlorophos-phates, etc. which are available, for example from commercial sources such as Sigma-Aldrich Chemical, or synthesized using known techniques.

Preferably the substituents are reacted in an inert solvent such as methylene chloride, chloroform, DMF or mixtures thereof. The reaction also preferably is conducted in the presence of a base, such as dimethylaminopyridine, diisopropylethylamine, pyridine, triethylamine, etc. to neutralize any acids generated and at a temperature from 0°C up to about 22°C (room temperature).

More particularly, one method of preparing a polymeric transport system includes reacting a compound of the formula (VIII):



(VIII)

wherein (v) and (t) are independently 0 or a positive integer up to about 6, and preferably about 1;

L_1 and L_2 are independently selected bifunctional linkers;

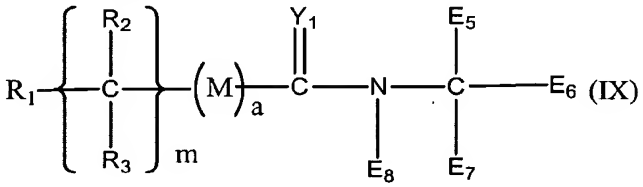
Y_{4-7} are independently selected from the group consisting of O, S and NR_{14};

R_{11-14} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

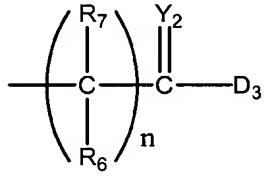
Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group; and

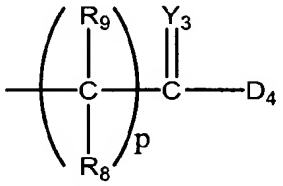
B'_1 is a residue of a hydroxyl- or an amine-containing moiety;

with a compound of the formula (IX):



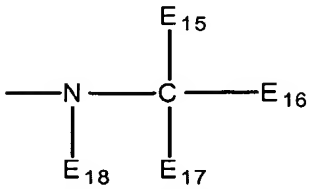
wherein

E_5 is 

E_{6-8} are independently H, E_5 or 

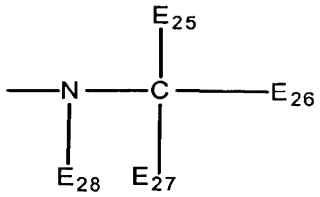
wherein D_3 and D_4 are independently OH, a leaving group which is capable of reacting with an unprotected amine or hydroxyl or a terminal branching group.

In further aspects of the method, D_3 and D_4 are independently selected terminal branching groups of the formula (X):



(X)

where E_{15-18} are selected from the same group which defines E_{5-8}, except that D_3 and D_4 are changed to D'_3 and D'_4, where D'_3 and D'_4 are independently OH, a moiety of formula (IV) or (V), or



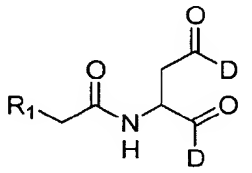
(XI)

wherein E_{25-28} are selected from the same group which defines E_{5-8}, except that D_3 and D_4 are changed to $D"_3$ and $D"_4$ which are defined as being independently OH or a leaving group which is capable of reacting with an unprotected amine or hydroxyl.

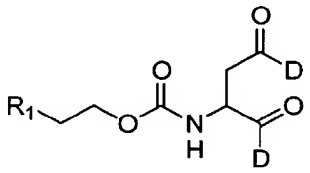
Such synthetic techniques allow up to sixteen (16) equivalents of carboxylic acid or activated carboxylic acid, for example, to be attached. As shown in the preferred structures herein, PEG residues with terminally branched multi-acids are preferred aspects of the invention.

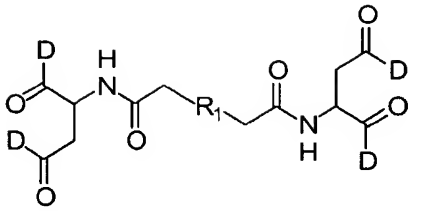
Returning to formula (IX), R_1 is a polymeric residue; Y_1 is O, S or NR_4; M is O, S or NR_5; (n) and (p) are independently 0 or a positive integer; Y_{2-3} are independently O, S or NR_{10}; and R_{2-10} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy.

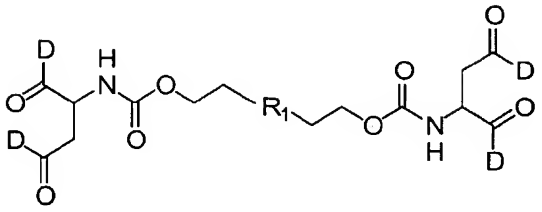
Regardless of the synthesis selected, some of the preferred compounds which result from the synthesis techniques described herein include:

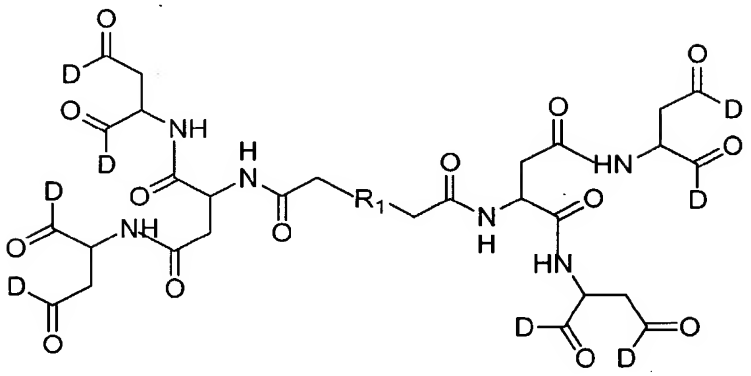


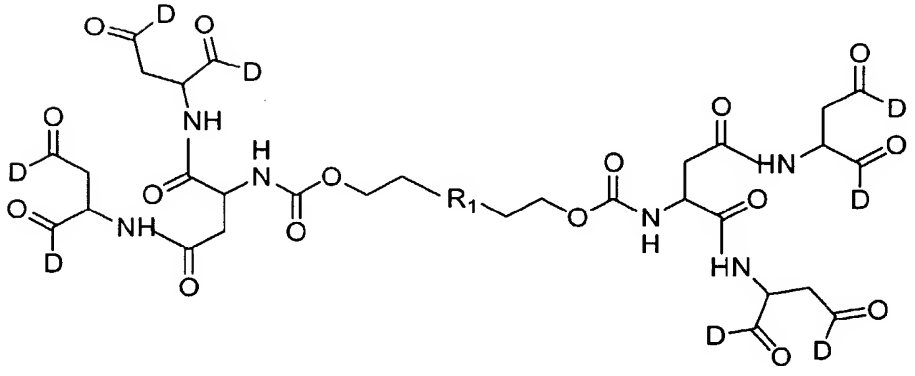
,

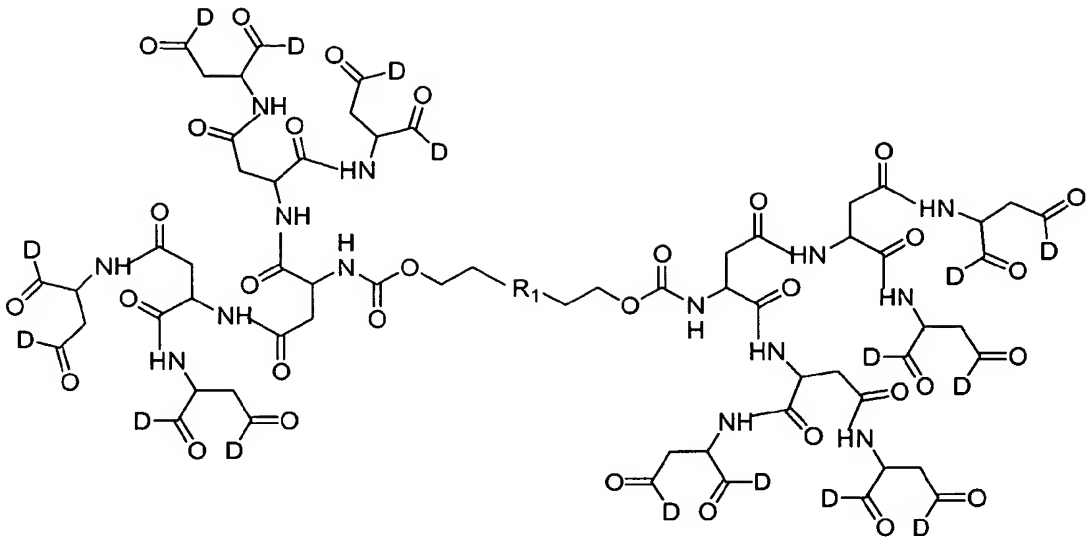




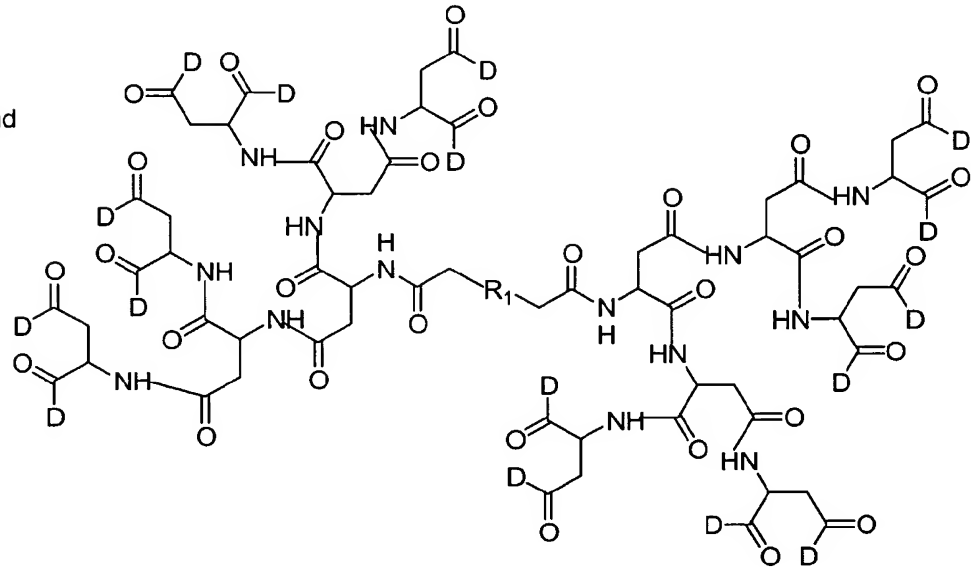




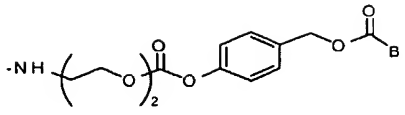


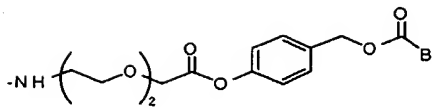


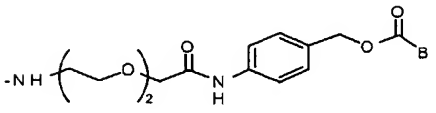
and

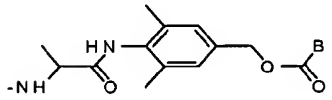


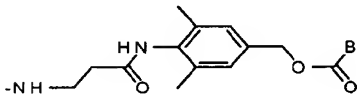
wherein R_1 is a polymer residue such as a PAO or PEG residue and D is OH, formula (IV) or (V). Preferably, D is

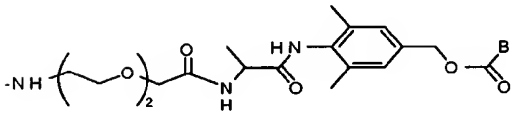


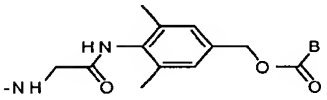


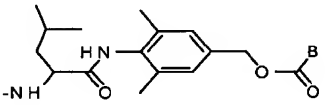


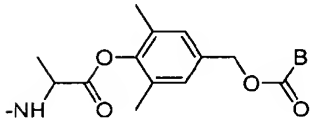


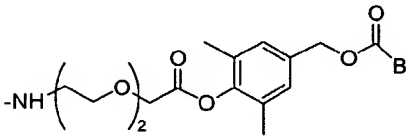


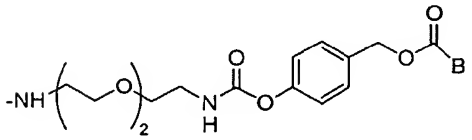


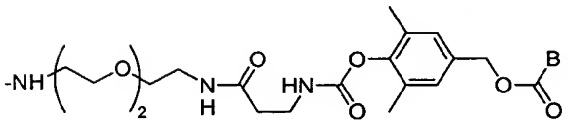


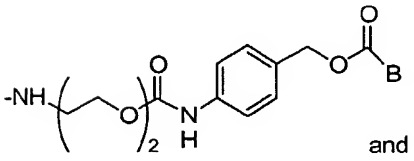




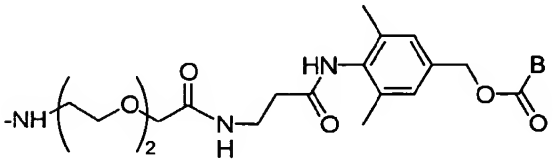






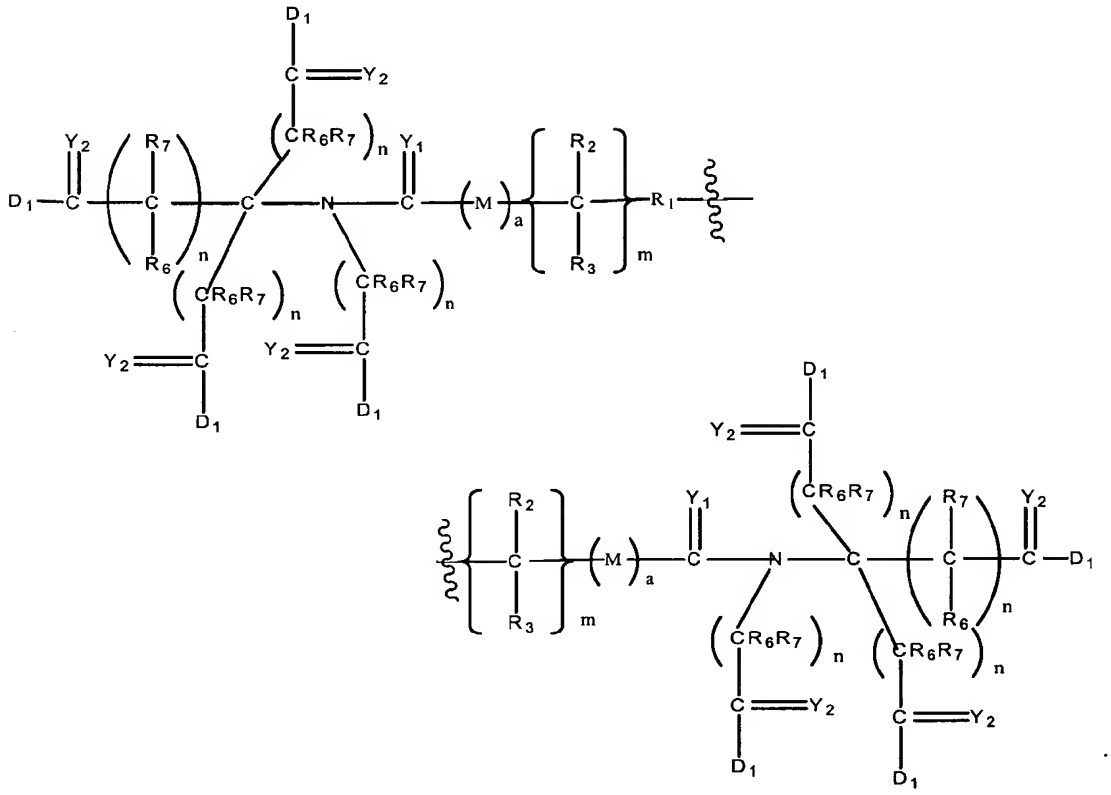


and



where B is a residue of an amine or a hydroxyl- containing drug.

In another preferred aspect of the invention, the compounds of the present invention are of formula (XII):



wherein all variables are as previously defined above.

G. *IN VIVO* DIAGNOSTICS

A further aspect of the invention provides the conjugates of the invention optionally prepared with a diagnostic tag linked to the transport enhancer described above, wherein the tag is selected for diagnostic or imaging purposes. Thus, a suitable tag is prepared by linking any suitable moiety, e.g., an amino acid residue, to any art-standard emitting isotope, radio-opaque label, magnetic resonance label, or other non-radioactive isotopic labels suitable for magnetic resonance imaging, fluorescence-type labels, labels exhibiting visible colors and/or capable of fluorescing under ultraviolet, infrared or electrochemical stimulation, to allow for imaging tumor tissue during surgical procedures, and so forth. Optionally, the diagnostic tag is incorporated into and/or linked to a

conjugated therapeutic moiety, allowing for monitoring of the distribution of a therapeutic biologically active material within an animal or human patient.

In a still further aspect of the invention, the inventive tagged conjugates are readily prepared, by art-known methods, with any suitable label, including, e.g., radioisotope labels. Simply by way of example, these include ^{131}Iodine, ^{125}Iodine, ^{99m}Technetium and/or ^{111}Indium to produce radioimmunoscintigraphic agents for selective uptake into tumor cells, *in vivo*. For instance, there are a number of art-known methods of linking peptide to Tc-99m, including, simply by way of example, those shown by U.S. Patent Nos. 5,328,679; 5,888,474; 5,997,844; and 5,997,845, incorporated by reference herein.

Broadly, for anatomical localization of tumor tissue in a patient, the conjugate tag is administered to a patient or animal suspected of having a tumor. After sufficient time to allow the labeled immunoglobulin to localize at the tumor site(s), the signal generated by the label is detected, for instance, visually, by X-ray radiography, computerized transaxial tomography, MRI, by instrumental detection of a luminescent tag, by a photo scanning device such as a gamma camera, or any other method or instrument appropriate for the nature of the selected tag.

The detected signal is then converted to an image or anatomical and/or physiological determination of the tumor site. The image makes it possible to locate the tumor *in vivo* and to devise an appropriate therapeutic strategy. In those embodiments where the tagged moiety is itself a therapeutic agents, the detected signal provides evidence of anatomical localization during treatment, providing a baseline for follow-up diagnostic and therapeutic interventions.

H. METHODS OF TREATMENT

Another aspect of the present invention provides methods of treatment for various medical conditions in mammals. The methods include administering to the mammal in need of such treatment, an effective amount of a prodrug, such as a multi-loaded Ara-C-PEG conjugates, which has been prepared as described herein. The compositions are useful for, among other things, treating neoplastic disease, reducing tumor burden, preventing metastasis of neoplasms and preventing recurrences of tumor/neoplastic growths in mammals.

The amount of the prodrug administered will depend upon the parent molecule included therein. Generally, the amount of prodrug used in the treatment methods is that amount which effectively achieves the desired therapeutic result in mammals. Naturally, the dosages of the various prodrug compounds will vary somewhat depending upon the parent compound, rate of in vivo hydrolysis, molecular weight of the polymer, etc. In general, however, prodrug taxanes are administered in amounts ranging from about 5 to about 500 mg/m^2 per day, based on the amount of the taxane moiety. Camptothecin prodrugs are also administered in amounts ranging from about 5 to about 500 mg/m^2 per day. The range set forth above is illustrative and those skilled in the art will determine the optimal dosing of the prodrug selected based on clinical experience and the treatment indication. Actual dosages will be apparent to the artisan without undue experimentation.

The prodrugs of the present invention can be included in one or more suitable pharmaceutical compositions for administration to mammals. The pharmaceutical compositions may be in the form of a solution, suspension, tablet, capsule or the like, prepared according to methods well known in the art. It is also contemplated that administration of such compositions may be by the oral and/or parenteral routes depending upon the needs of the artisan. A solution and/or suspension of the composition may be utilized, for example, as a carrier vehicle for injection or infiltration of the composition by any art known methods, e.g., by intravenous, intramuscular, subdermal injection and the like.

Such administration may also be by infusion into a body space or cavity, as well as by inhalation and/or intranasal routes. In preferred aspects of the invention, however, the prodrugs are parenterally administered to mammals in need thereof.

I. EXAMPLES

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective scope of the invention. The underlined and bold-faced numbers recited in the Examples correspond to those shown in the figures. It is also to be understood that the bifunctional PEG derivatives (alpha and omega substituted) are used and that the figures show only one side of the compounds actually formed in light of the mirror images of the terminal groups.

General. All reactions were run under an atmosphere of dry nitrogen or argon. Commer-

cial reagents were used without further purification. All PEG compounds were dried under vacuum or by azeotropic distillation (toluene) prior to use. ^{1}H spectra were obtained with a JEOL FT NMR System JNM GSX-270 or Varian MercuryVX-300 instrument using deuteriochloroform as solvent unless specified. ^{13}C NMR spectra were obtained at 67.80 MHz on the JNM GSX-270 or 75.46 MHz on the Varian MercuryVX-300. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) and coupling constants (*J* values) are given in hertz (Hz). All PEG conjugated compounds were dissolved (~15 mg/mL) in sterile saline (0.9%) for injection prior to *in vivo* drug treatments and were given as their ara-C equivalents (absolute amt. of ara-C given).

HPLC Method. Analytical HPLC's were performed using a C8 reversed phase column (Beckman, ultrasphere) under isocratic conditions with an 80:20 mixture (v/v) of methanol-water as mobile phase. Peak elutions were monitored at 254 nm using a UV detector. To detect the presence of any free PEG and also to confirm the presence of PEGylated product, an evaporative light scattering detector (ELSD), Model PL-EMD 950 (Polymer Laboratories), was employed. Based on ELSD and UV analysis, all the final PEGylated products were free of native drug and were ≥ 95% pure by HPLC.

Analysis of Ara-C Content in PEG Derivatives. For the determination of the ara-C content in PEG derivatives, *N*^{4}-acetylcytidine was used as the basis because of the absorbance change due to the acylation of ara-C. The UV absorbance of *N*^{4}-acetylcytidine in H_2O was determined at 257 nm for six different concentrations ranging from 0.01 μmol/mL to 0.05 μmol/mL. From the standard plot of absorbance *vs.* concentration, the absorption coefficient, ε, of *N*^{4}-acetylcytidine was calculated to be 36.4 (O.D. at 257 nm for 1 mg/mL with 1.0 cm light path). PEGylated ara-C derivatives were dissolved in H_2O at an approximate concentration of 0.015 μmol/mL (based on a MW of 40 kDa) and the UV absorbance of these compounds at 257 nm was determined. Using this value and employing the absorption coefficient, ε, obtained from the above, the concentration of ara-C in the sample was determined. Dividing this value by the sample concentration provided the percentage of ara-C in the sample.

Analysis of Melphalan Content in PEG Derivatives. For the determination of the melphalan content in PEG derivatives, melphalan was used as a standard. The UV absorbance of melphalan in DMF-H_2O (9:1, v/v) was determined at 264 nm for 5 different

concentrations ranging from 0.02 μmol/mL to 0.06 μmol/mL. From the standard plot of absorbance *vs.* concentration, the absorption coefficient, ε, of melphalan was calculated to be 54.6 (O.D. at 264 nm for 1 mg/mL with 1.0 cm light path). PEGylated melphalan derivatives were dissolved in DMF-H_2O (9:1, v/v) at an approximate concentration of 0.013 μmol/mL (based on a MW of 40 kDa) and the UV absorbance of these compounds at 264 nm was determined. Using this value and employing the absorption coefficient, ε, obtained from the above, the concentration of melphalan in the sample was determined. Dividing this value by the sample concentration provided the percentage of melphalan in the sample.

Abbreviations. DCM (dichloromethane), DIEA (*N,N*-diisopropylethylamine), DMAP (4-(dimethylamino)pyridine), DSC (*N,N*-disuccinimidyl carbonate), EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), HOBT (1-hydroxybenzotriazole), IPA (2-propanol), NMM (*N*-methylmorpholine), TBDMS-Ci (*tert*-butyldimethylsilyl chloride), TEA (triethylamine), TFA (trifluoroacetic acid).

Example 1.

4-(*tert*-Butyldimethylsilanyloxymethyl)-2,6-dimethyl-phenol (2). A solution of triethylamine (5 mL, 35.87 mmol) in DCM (5 mL) was slowly added over 1 hour to a solution of 4-hydroxy-3,5-dimethylbenzyl alcohol (**1**, 1.0 g, 6.58 mmol) and TBDMS-Cl (1.61 g, 10.7 mmol) in DCM (10 mL) at 0 °C. The final solution was allowed to warm to room temperature and stirred overnight at room temperature. The solvent was removed *in vacuo*. The residue was dissolved in DCM and washed with water four times to give 1.5 g (86%) of **2**: ^{1}H NMR δ_0.00 (s, 6H, Si(CH_3)$_2$), 0.84 (s, 9H, SiC(CH_3)$_3$), 2.11 (s, 6H, 2 × Ar-CH_3), 4.50 (s, 3H, Ar-CH_2OH), 6.82 (s, 2H, 2 × Ar-C*H*); ^{13}C NMR (67.80 MHz, $CDCl_3$) δ -3.25, 15.91, 18.48, 26.01, 64.91, 122.84, 126.93, 132.85, 151.15.

Example 2.

2-*tert*-Butoxycarbonylamino-propionic acid 4-(tert-butyl-dimethylsilanyloxy-methyl)-2,6-dimethyl-phenyl ester (3). A mixture of Boc-Ala-OH (1.279 g, 6.77 mmol), **2** (1.2 g, 4.511 mmol), EDC•HCl (1.299 g, 6.77 mmol), and DMAP (825.6 mg, 6.77 mmol) in DCM (50 mL) was stirred at 0 °C and then allowed to warm to room temperature overnight. The solution was washed with 0.5N HCl (25 mL × 4), dried over anhyd $MgSO_4$, and the solvent removed *in vacuo* to give 1.95 g (99%) of **3**: ^{1}H NMR δ 0.00 (s, 6H, Si(CH_3)$_2$), 0.84 (s, 9H, SiC(CH_3)$_3$), 1.36 (s, 9H, OC(CH_3)$_3$), 1.50 (d, 3H, *J* =

0.81 Hz, $CHCH_3$), 2.07 (s, 6H, 2 × Ar-CH_3), 4.51 (m, 1H, $CHCH_3$), 4.56 (s, 1H, Ar-CH_2O), 6.91 (s, 2H, 2 × Ar-CH); ^{13}C NMR δ -3.55, 16.34, 18.39, 18.53, 25.92, 28.26, 49.34, 64.43, 77.46, 126.28, 129.68, 138.97, 146.57, 155.17, 171.43.

Example 3.

2-*tert*-Butoxycarbonylamino-propionic acid 4-hydroxymethyl-2,6-dimethyl-phenyl ester (4). Compound **3** (1.9 g, 4.35 mmol) was dissolved in acetic acid (25 mL), THF (8 mL), and water (8 mL) and the solution was stirred at room temperature for 1 h. The solvent was removed *in vacuo* and the residue was dissolved in DCM (100 mL), washed with 0.5% $NaHCO_3$ (50 mL) and water (50 mL). The organic layer was dried over anhyd $MgSO_4$ and the solvent removed *in vacuo*. The residue was purified by silica gel column chromatography to give 0.85 g (55%) of **4**: ^1H NMR δ 1.46 (s, 9H, $OC(CH_3)_3$), 1.58 (d, 3H, J = 0.81 Hz, $CHCH_3$), 2.13 (s, 6H, 2 × Ar-CH_3), 4.51 (m, 1H, $CHCH_3$), 4.56 (s, 1H, Ar-CH_2O), 7.03 (s, 2H, 2 × Ar-CH); ^{13}C NMR (67.80 MHz, $CDCl_3$) δ 16.17, 18.31, 28.18, 49.30, 64.39, 79.98, 127.12, 129.99, 138.68, 146.95, 155.14, 171.38.

Example 4.

2-*tert*-Butoxycarbonylamino-propionic acid 4-(2,5-dioxo-pyrrolidin-1-yloxy-carbonyloxymethyl)-2,6-dimethyl-phenyl ester (5). A mixture of **4** (730 mg, 2.26 mmol), DSC (752.1 mg, 2.94 mmol), and pyridine (232.1 mg, 2.94 mmol) in anhydrous chloroform (20 mL) was stirred at 25-30 °C overnight. The reaction mixture was washed with 0.5N HCl (15 mL) and the organic layer dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* to give **5** as a solid (899.5 mg, 86%): ^{13}C NMR δ 16.32, 18.45, 25.45, 28.28, 49.35, 72.25, 77.21, 80.10, 128.99, 129.02, 130.94, 130.91, 148.45, 151.04, 155.14, 168.57.

Example 5.

2-*tert*-Butoxycarbonylamino-propionic acid 4-[1-(3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-2-oxo-1,2-dihydro-pyrimidin-4-ylcarbamoyloxymethyl]-2,6-dimethyl-phenyl ester (7). A mixture of **5** (760 mg, 1.64 mmol), ara-C (**6**, 996 mg, 4.1 mmol) and DIEA (634.3 mg, 4.92 mmol) in anhydrous DMF (40 mL) was stirred overnight at 40°C. The reaction mixture was concentrated *in vacuo* and purified by silica gel column chromatography. The yield of pure product (**7**) was 200 mg (21%): ^{13}C NMR (CD_3OD + CDCl3) δ 16.45, 17.61, 28.61, 50.41, 62.38, 67.74, 75.98, 77.75, 80.49, 86.77, 88.96, 95.68, 129.41, 131.48, 134.29, 147.18, 148.96, 154.14, 157.29, 157.43, 164.27,

172.98.

Example 6.

2-Amino-propionic acid 4-[1-(3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-2-oxo-1,2-dihydro-pyrimidin-4-ylcarbamoyloxymethyl]-2,6-dimethyl-phenyl ester (8) TFA salt. Compound **7** (200 mg, 0.34 mmol) was stirred in DCM (4 mL) and TFA (2 mL) for 2 h at room temperature and the solvent was removed *in vacuo*. The residue was recrystallized from DCM-ether to give 200 mg (100%) of **8**: ^{13}C NMR (CD_3OD) δ 16.28, 16.44, 49.86, 62.74, 67.97, 76.53, 78.08, 87.47, 89.47, 95.76, 129.96, 131.70, 135.76, 148.00, 148.89, 154.58, 164.68, 169.62.

Example 7.

Compound 10. EDC•HCl (131.1 mg, 0.68 mmol) was added to a mixture of PEG-aspartic acid (**9**, mw. 40,000, 1.73 g, 0.043 mmol), **8** (210 mg, 0.43 mmol), NMM (137.9 mg, 1.37 mmol), and HOBT (69.1 mg, 0.51 mmol) in anhydrous DCM (25 mL) and DMF (15 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and then allowed to warm to room temperature overnight. The solvent was removed and the residue was recrystallized twice from IPA to yield 1.4 g (82%) of **10**. The amount of ara-C present in the product measured by UV assay was 2.26 wt%: ^{13}C NMR (D_2O) δ 14.51, 18.19, 18.68, 33.67, 45.20, 52.46, 62.92, 63.44, 72-11-72.19 (PEG), 74.32, 77.64, 78.36, 87.15, 89.17, 95.46, 131.04, 133.19, 135.97, 148.93, 150.04, 155.76, 158.71, 165.46, 173.25, 174.46, 187.43.

Example 8.

4-(*tert*-Butyl-dimethyl-silanyloxymethyl)-phenol (12). A solution of 4-hydroxymethyl phenol (**11**, 9.3 g, 75 mmol) in DMF (50 mL) was sparged with anhydrous nitrogen gas for 10 min, followed by addition of TBDMS-Cl (12.44 g, 82 mmol). The reaction mixture was cooled to 0 °C and then a solution of TEA (30.36 g, 300 mmol) in DMF (25 mL) was added slowly. The reaction solution was stirred overnight at room temperature and concentrated *in vacuo*. The residue was partitioned twice between water (100 mL) and DCM (200 mL). The organic layers were combined and dried over anhydrous $MgSO_4$ followed by removal of the solvent *in vacuo* to give **12** (16.3 g, 91%): ^{13}C NMR δ -5.344 (2 × Si-CH_3), 18.285 (Si-$C(CH_3)_3$), 25.850 (Si-$C(CH_3)_3$), 64.840 (Ar-CH_2O), 115.221 (Ar-C^2), 127.842 (Ar-C^3), 132.962 (Ar-C^4), 155.248 (Ar-C^1).

Example 9.

Compound 14. Pyridine (983 mg, 12.43 mmol) was added to a suspension of **12** (2.7 g,

11.3 mmol) and DSC (3.18 g, 12.43 mmol) in $CHCl_3$ (140 mL) and the mixture was refluxed overnight. After cooling to room temperature, **13** (3.398 g, 13.7 mmol) was added to the solution and the reaction mixture was stirred at room temperature overnight. The mixture was washed with 0.1N HCl (3 × 100 mL) and brine (100 mL). The organic layer was dried over anhydrous $MgSO_4$ and the solvent removed *in vacuo*. The residue was dissolved in hexane (100 mL) followed by filtration of insoluble impurities. The hexane filtrate was concentrated to give product **14** (5.1 g, 88%): ^{13}C NMR δ -5.469 (2 × Si-CH_3), 18.235 (Si-$C(CH_3)_3$), 25.790 (Si-C(CH_3)$_3$), 28.263 (O-C(CH_3)$_3$), 40.230 (CH_2NH), 40.917 (CH_2NH), 64.392 (Ar-CH_2O), 69.892 (CH_2O), 70.192 (CH_2O), 70.253 (CH_2O), 79.305 ($OC(CH_3)_3$), 121.371 (Ar-C^2), 126.943 (Ar-C^3), 138.482 (Ar-C^4), 149.960 (O*C*(=O)NH), 154.936 (O*C*(=O)NH), 156.096 (Ar-C^1).

Example 10.

Compound 15. Compound **14** (5 g, 9.76 mmol) was dissolved in acetonitrile (30 mL) and water (30 mL) followed by the addition of HOAc (90 mL). The reaction mixture was stirred at room temperature for 1.5 h followed by the removal of the solvent. The residue was dissolved in DCM (300 mL), washed with water (3 × 300 mL) and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* to give **15** (4.1 g, 80%): ^{13}C NMR δ 28.233 (OC(CH_3)$_3$), 40.184 (CH_2NH), 40.871 (CH_2NH), 64.484 (Ar-CH_2O), 69.811-70.191 (4 × CH_2O), 79.228 ($OC(CH_3)_3$), 121.661 (Ar-C^2), 127.950 (Ar-C^3), 138.177 (Ar-C^4), 150.418 (O*C*(=O)NH), 154.875 (O*C*(=O)NH), 156.111 (Ar-C^1).

Example 11.

Compound 16. A solution of compound **15** (800 mg, 2.01 mmol) and DSC (726 mg, 2.8 mmol) in DCM (80 mL) was cooled to 0 °C followed by the addition of pyridine (224 mg, 2.8 mmol). The solution was stirred overnight at 0-5 °C followed by the removal of solvent *in vacuo*. The residue was dissolved in DCM (40 mL), washed with water (2 × 20 mL), dried over anhydrous $MgSO_4$ and the solvent removed *in vacuo* to give **16** (0.88 g, 88%): ^{13}C NMR δ 24.852 (NHS's 2 × C(=O)CH_2), 27.834 (OC(CH_3)$_3$, 39.777 (CH_2NH), 40.443 (CH_2NH), 69.230-69.640 (4 × CH_2O), 71.624 (Ar-CH_2O), 78.690 ($OC(CH_3)_3$), 121.583 (Ar-C^2), 129.468 (Ar-C^3), 137.110 (Ar-C^4), 148.092 (O*C*(=O)O), 151.203 & 154.134 (O*C*(=O)NH), 155.772 (O*C*(=O)NH), 168.752 (Ar-C^1), 172.809 (NHS's 2 × C(=O)CH_2).

Example 12.

Compound 17. Compound **16** is subjected to similar conditions as shown in Example 6-8 to give **19**.

Example 13.

Compound 22. PEG diol (**20**, 55 g, 1.38 mmol) was azeotroped in toluene over a 2 h period followed by the removal of 200 mL of solvent by rotary evaporation. The solution was cooled to ~30 °C and triphosgene (0.544 g, 1.83 mmol) was added as a solid followed by the addition of anhydrous pyridine (0.434 g, 5.49 mmol). The reaction mixture stirred at 50 °C for 1 h. *N*-hydroxyphthalimide (**21**, 1.12 g, 6.88 mmol) and anhydrous pyridine (0.54 g, 6.88 mmol) were added to the chloroformate mixture and the reaction stirred for a further 2 h at 50 °C and then for 12 h at room temperature. The reaction mixture was filtered through filter paper and the solvent removed *in vacuo*. The product was recrystallized from DCM-ethyl ether (1100 mL, 8:2, v/v) to give the product (50.9 g, 92%): ^{13}C NMR δ 123.62, 128.10, 134.55, 152.00, 160.00.

Example 14.

PEG-cmc-Asp-O-*t*-Bu (24). Compound **22** (mw. 40,000, 20 g, 0.459 mmol) and aspartic acid di *t*-butyl ester•HCl (**23**, 1.0 g, 3.55 mmol) were dissolved in anhydrous DCM, followed by the addition of DMAP (0.433 g, 3.55 mmol). The solution was refluxed overnight followed by precipitation by addition of ethyl ether (1 L). The solid was isolated by filtration and recrystallized from IPA (1 L) twice. The filter cake was washed with IPA (200 mL) and ether (200 mL) to give 15.6 g (78%) of product after drying at 45 °C *in vacuo*: ^{13}C NMR δ 27.837 ($CH_2CO_2C(CH_3)_3$), 27.991 ($CHCO_2C(CH_3)_3$), 37.752 ($CHCH_2CO_2$), 50.800 (NH*C*H), 64.212 ($OCH_2CH_2OC(=O)NH$), 81.333 ($CH_2CO_2C(CH_3)_3$), 82.007 ($CHCO_2C(CH_3)_3$), 155.924 ($OCH_2CH_2OC(=O)NH$), 169.674 ($CH_2CO_2C(CH_3)_3$), 169.969 ($CHCO_2C(CH_3)_3$).

Example 15.

PEG-cmc-Asp-OH (25). Compound **24** (15 g, 0.375 mmol) was dissolved in DCM (150 mL) followed by addition TFA (75 mL). The solution was stirred at room temperature for 2 h and hexane (500 mL) was added to precipitate the solid. The solid was triturated with hexane to remove TFA followed by recrystallization from chilled DCM-ether. The recrystallized solid was redissolved in DCM (150 mL) and washed with water (150 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, concentrated *in vacuo*,

and precipitated with ether to give 12.4 g (83%) of product: ^{13}C NMR δ 36.441 ($CHCH_2CO_2$), 50.177 ($NHCH$), 64.390 ($OCH_2CH_2OC(=O)NH$), 81.333 ($CH_2CO_2C(CH_3)_3$), 82.007 ($CHCO_2C(CH_3)_3$), 156.172 ($OCH_2CH_2OC(=O)NH$), 171.944 ($CH_2CO_2C(CH_3)_3$), 172.211 ($CHCO_2C(CH_3)_3$).

Example 16.

Melphalan Me ester (27). Melphalan (**26**, 1.00 g, 3.28mmol) was suspended in 2,2 dimethoxypropane (65.59 mL, 533.49 mmol). To the suspension was added aqueous HCl (36 %, 3.28 mL) and absolute methanol (4 mL). The mixture was warmed to mild reflux with vigorous stirring until the solution started to turn slightly brown. The mixture then was stirred at room temperature for 18 hours. The reaction mixture was concentrated *in vacuo* and the crude product precipitated from the residue with ether. The solid was filtered, washed with ether, and purified by silica gel column chromatography ($CHCl_3$: MeOH = 9:1, v/v) to yield the desired product (0.47g, 45%): ^{13}C NMR δ 39.751, 40.340, 51.912, 53.435, 55.803, 112.124, 126.076, 130.620, 145.033, 175.754.

Example 17.

Compound 28. A mixture of **16** (1.91 g, 3.55 mmol), **27** (1.7 g, 5.33 mmol), and DMAP (0.519 g, 4.25 mmol) in anhydrous chloroform (30 mL) was stirred at room temperature overnight under nitrogen and then concentrated *in vacuo.* The residue was redissolved in DCM (100 mL), and washed three times with 1N HCl (50 mL). The organic layer was dried over magnesium sulfate, concentrated, and purified by silica gel column chromatography (EtOAc:Hexane = 8:2, v/v) to yield the desired product (0.8301 g, 32 %): ^{13}C NMR δ 28.173, 36.571, 40.076, 40.680, 51.838, 52.956, 54.381, 54.933, 65.707, 68.390, 69.212, 69.610, 76.056, 76.480, 76.891, 78.547, 111.060, 120.575, 123.285, 128.087, 128.318, 129.333, 131.002, 132.029, 143.856, 149.544, 149.801, 153.127, 154.193, 154.565, 170.539.

Example 18.

Compound 29. TFA (2.5 mL) was slowly added to a solution of **28** (0.8306 g, 1.12 mmol) in DCM (5 mL). The reaction solution was stirred at room temperature for 1.5 hours, followed by concentration *in vacuo.* The residue was recrystallized with DCM-ether to yield the desired product (0.0838 g, 13 %): ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 37.218, 39.814, 40.715, 41.196, 52.521, 53.625, 55.091, 66.349, 66.635, 69.998, 70.107, 111.768, 121.357, 123.995, 128.781, 130.062, 132.927, 144.615, 150.218, 154.381,

154.988, 171.411.

Example 19.

Compound 30. EDC (0.048 g, 0.253 mmol) and DMAP (0.077 g, 0.632 mmol) were added to a mixture of PEG-cmc-Asp-COOH (**25**, 1.26 g, 0.032 mmol) and **29** (0.191 g, 0.253 mmol) in anhydrous DCM (20 mL) and anhydrous DMF (5 mL) at 0 °C in an ice bath. Reaction mixture was stirred at room temperature overnight under nitrogen and then concentrated *in vacuo*. The residue was recrystallized from DCM-ether and from IPA to yield the desired product (0.874 g, 64.2 %). The amount of melphalan in the product measured by UV assay was 2.4% wt/wt: ^{13}C NMR δ 36.385, 38.995, 40.090, 40.606, 51.809, 52.949, 54.506, 61.039, 62.451, 65.683, 68.300-72.780 (PEG), 111.372, 120.942, 123.754, 128.474, 129.733, 132.185, 144.332, 150.106, 153.868, 154.768, 160.141, 171.165.

Example 20.

Compound 32. A solution of **31** (2.0 g, 5.78 mmol, 1 eq), **2** (3.1 g, 11.56 mmol, 2 eq), and DMAP (1.76 g, 14.45 mmol, 2.5 eq) in anhydrous DCM (20 mL) was refluxed under nitrogen for 4 hours and then cooled to room temperature. The solution was diluted with DCM (100 mL) and washed three times with water (50 mL). The organic layer was dried over anhydrous magnesium sulfate, concentrated, and purified by silica gel column chromatography (5-25 % MeOH in DCM, v/v) to yield the desired product (2.50 g, 87 %): ^{13}C NMR δ -5.421, 15.994, 18.324, 25.850, 28.282, 40.251, 64.392, 67.413, 68.641, 70.331, 79.304, 126.409, 129.878, 139.171, 147.209, 153.200, 156.041.

Example 21.

Compound 33. Compound **32** (2.5 g, 5.027 mmol) was dissolved in acetonitrile (8 mL) and water (8 mL) followed by addition of HOAc (23 mL). The reaction mixture was stirred at room temperature for 1.5 h, followed by the removal of the solvent. The residue was dissolved in DCM (50 mL), washed with 0.5% sodium bicarbonate (3 × 25 mL) and then with water (25 mL). The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed *in vacuo* to give **33** (1.556 g, 80.8%): ^{13}C NMR δ 15.828, 28.206, 40.187, 53.320, 64.417, 67.489, 68.513, 70.241, 79.278, 82.760, 113.173, 127.279, 130.159, 138.915, 147.580, 153.020, 156.067.

Example 22.

Compound 34. Pyridine (0.427 mL, 5.277 mmol) was added to a solution of compound

33 (1.556 g, 4.059 mmol) and DSC (1.352 g, 5.277 mmol) in anhydrous chloroform (31.12 mL). The solution stirred overnight at room temperature, followed by the removal of solvent *in vacuo*. The reaction mixture was diluted to 50 mL with chloroform, washed with 0.5N HCl three times, dried over anhydrous $MgSO_4$, and the solvent removed *in vacuo* to give **34** (1.722 g, 81%): ^{13}C NMR δ 15.857, 25.256, 28.210, 40.143, 67.568, 68.507, 70.270, 72.099, 79.249, 129.130, 130.959, 131.094, 148.918, 151.654, 152.845, 156.034, 168.773.

Example 23.

Compound 35. A mixture of **34** (1.37 g, 2.61 mmol), **27** (1.249 g, 3.91 mmol), and DMAP (0.382 g, 3.13 mmol) in anhydrous chloroform (40 mL) was stirred at room temperature overnight under nitrogen and then concentrated *in vacuo*. The residue was redissolved in DCM (100 mL), and washed three times with 1N HCl (50 mL). The organic layer was dried over magnesium sulfate and concentrated to yield the desired product (1.85 g, 97%): ^{13}C NMR δ 15.981, 28.321, 36.820, 39.943, 40.315, 52.244, 53.192, 53.358, 54.804, 66.325, 67.541, 68.539, 70.293, 79.266, 111.983, 112.329, 124.310, 128.610, 130.338, 130.479, 131.081, 133.961, 145.084, 148.028, 152.828, 155.542, 155.875, 172.041.

Example 24.

Compound 36. TFA (4.6 mL) was slowly added to a solution of **35** (1.85 g, 2.536 mmol) in DCM (10 mL). The reaction solution was stirred at room temperature for 1.5 hours, followed by concentration *in vacuo*. The residue was recrystallized with DCM-ether to yield the desired product (1.335 g, 71 %): ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 15.905, 36.846, 39.431, 40.379, 52.321, 53.396, 54.932, 66.273, 66.670, 67.374, 68.936, 112.021, 124.386, 128.585, 130.364, 130.505, 134.204, 145.161, 148.003, 152.995, 155.644, 172.259.

Example 25.

Compound 37. EDC (0.345 g, 1.79 mmol) and DMAP (0.77 g, 6.29 mmol) were added to a mixture of PEG-cmc-Asp-COOH (**25**, 7.25 g, 0.179 mmol) and **36** (1.34 g, 1.79 mmol) in anhydrous DCM (100 mL) and anhydrous DMF (25 mL) at 0 °C in an ice bath. The reaction mixture was stirred at room temperature overnight under nitrogen and concentrated *in vacuo*. The residue was recrystallized from DCM-ether and from IPA to yield the desired product (5.10 g, 66.3%). The amount of melphalan in the product

measured by UV assay was 2.85 % wt/wt: ^{13}C NMR δ 5.498, 36.214, 38.855, 39.894, 51.706, 52.815, 54.444, 60.947, 62.394, 63.756-73.461 (PEG), 111.438, 123.937, 128.080, 129.892, 133.572, 144.583, 147.462, 152.209, 155.116, 160.467, 170.411, 171.535.

Example 26

In vitro and in vivo data for compound 10.

In this Example, in vivo and in vitro data are presented and compared to unmodified Ara-C.

In Vivo

Athymic nude mice were implanted subcutaneous with a 4-5 mm^3 tissue fragment of LX-1 collected from donor mice. The tumor trocar site was observed twice weekly and measured once palpable. The tumor volume for each mouse was determined by measuring two dimensions with calipers and calculated using the formula: tumor volume = (length x $width^2$)/2. When tumors reached the average volume of 90 mm3, the mice were divided into their experimental groups which consisted of unmodified Ara-C and PEG-Ara-C **(Compound 10)**. The mice were sorted to evenly distribute tumor size, grouped into 4 to 6 mice/group, and ear punched for permanent identification. Drugs were administered intravenously q3d x 4 (Day 1, 4, 7 and 10) via the tail vein at an approximate rate of 0.5 mL per minute. Compounds were given both at an equal molar basis (absolute amount of active) of 20 mg/kg and at close their respective MTD (Ara-C, 100 mg/kg/dose (toxicity); 10, 40 mg/kg/dose (volume). Mouse weight and tumor size were measured at the beginning of study and twice weekly through week 4. Drug effectiveness was determined by comparing tumor growth in treated versus untreated (no vehicle) control mice. Five types of endpoints were used as the basis for comparison: (a) mean tumor volumes at Day 28; (b) mean percent change in individual tumor volumes from initial; (c) percent difference in tumor volume (%T/C), measured when the control group's median tumor volume reached approximately 800 - 1100 mm3 (exponential growth phase); (d) percent difference in tumor volume (%T/C) at Day 21 (~2000 mm3) and (e) the number of tumor regression (smaller tumor volume on Day 28 compared to Day 1) per group.

Results

Compound **10** demonstrated about equivalent antitumor activity with native Ara-C at only

20% of the active parent compound's dose.

Compound	½ (h)[a] Rat Plasma	IC50 (nM)[a] P388/O	LX-1 %T/C[b]
Ara-C	-	10	74.0 (100 mg / kg)
Compound 10	14	448	68.2 (20 mg / kg)

[a] All experiments were done at 37 °C in duplicate and t1/2 was measured by the disappearance of PEG derivatives. Standard deviation of measurements = ±10 %.

[b] Mean baseline tumor volume was 1000 mm3.

IN VITRO BIOASSAY

A series of in vitro assays were conducted to determine the IC_{50} for unmodified Ara-C and compound 10 using the P388/O (murine lymphoid neoplasm, Southern Research Institute) cell line. The P388/0 cells were grown in RPMI 1640 medium (Whittaker Bioproducts, Walkersville, Maryland) + 10% FBS (Hyclone Inc., Logan UT). Bioassays were performed in their respective media containing antibiotics and fungizone.

Ara-C was dissolved in DMSO and diluted to the appropriate concentration in culture media. The PEG-Ara-C was dissolved in water and diluted to the appropriate concentrations in culture media.

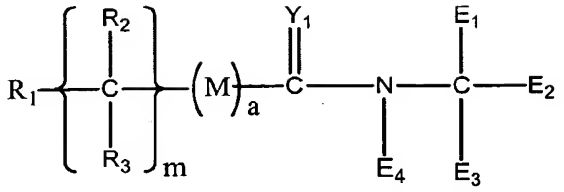
The assays were performed in duplicate in 96-well microtiter cell culture plates. Two fold serial dilution of the compounds were done in the microtiter plates. Cells were detached by incubating with 0.1% Trypsin/Versene at 37°. Trypsin was inactivated by adding the appropriate media for each cell line containing 10% FBS. To each well of the microtiter plates, 10,000 cells were added. After three days, cell growth was measured by addition of a metabolic indicator dye, Alamar Blue, according to the manufacturer's protocol. The IC_{50} value for the test compound and reference compound are provided above in the Table.

While there have been described what are presently believed to be the preferred embodiments of the invention, those skilled in the art will realize that changes and modifications may be made without departing from the spirit of the invention. It is intended to claim all such changes and modifications as fall within the true scope of the invention.

WHAT IS CLAIMED IS:

1. A compound comprising the formula:

(I)



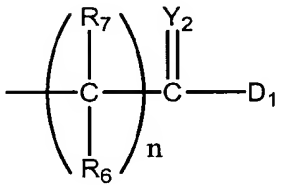
wherein:

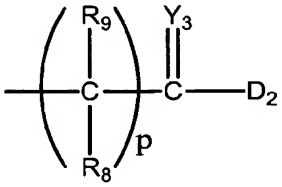
R_1 is a polymeric residue;

Y_1 is O, S or NR_4;

M is O, S or NR_5;

E_1 is





E_{2-4} are independently H, E_1 or

(a) is zero or one;

(m) is zero or a positive integer;

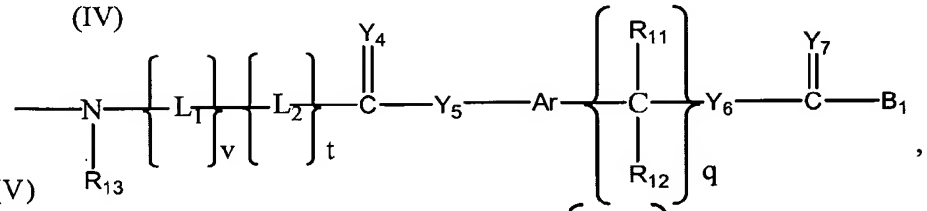
(n) and (p) are independently 0 or a positive integer;

Y_{2-3} are independently O, S or NR_{10};

R_{2-10} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

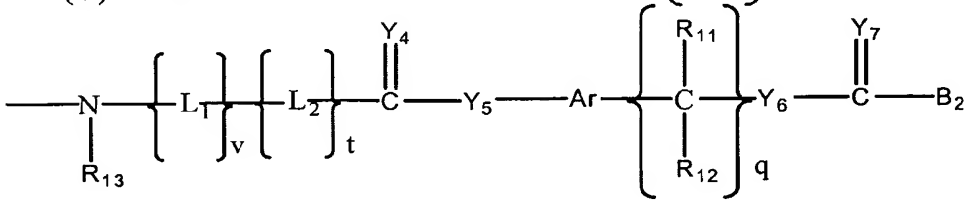
D_1 and D_2 are independently OH,

(IV)



,

(V)



or a terminal branching group;

wherein (v) and (t) are independently 0 or a positive integer up to about 6;

(q) is zero or a positive integer;

L_1 and L_2 are independently selected bifunctional linkers;

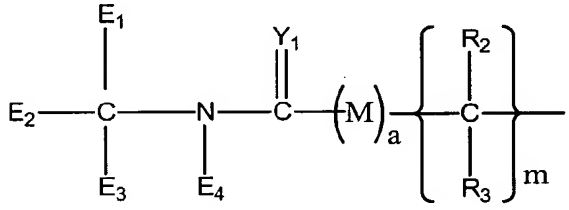
Y_{4-7} are independently selected from the group consisting of O, S and NR_{14};

R_{11-14} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroakoxy;

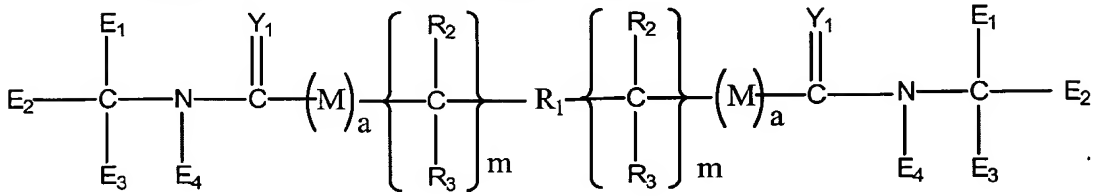
Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group;

B_1 and B_2 are independently selected from the group consisting of leaving groups, OH, residues of hydroxyl-containing moieties or amine-containing moieties.

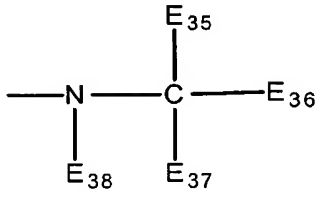
2. The compound of claim 1, wherein R_1 further comprises a capping group A, selected from the group consisting of hydrogen, NH_2, OH, CO_2H, C_{1-6} moieties and



3. A compound of claim 2, comprising the formula:

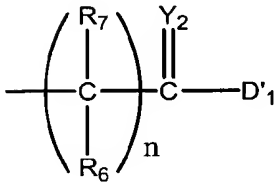


4. The compound of claim 1, wherein said terminal branching group comprises the formula:

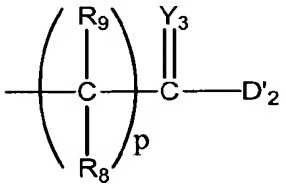


wherein

E_{35} is



E_{36-38} are independently H, E_{35} or



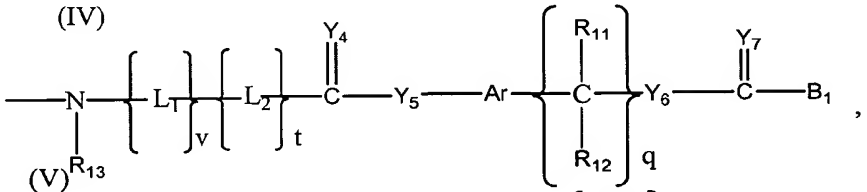
(n) and (p) are independently 0 or a positive integer;

Y_{2-3} are independently O, S or NR_{10};

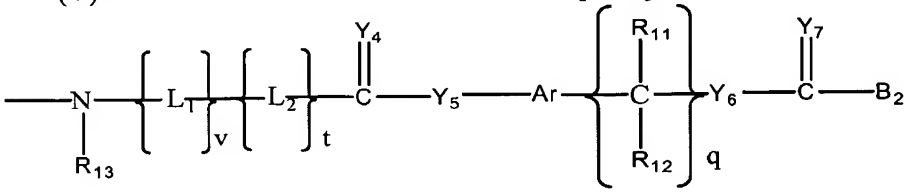
R_{6-10} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

D'_1 and D'_2 are independently OH,

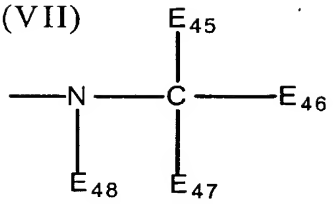
(IV)



(V)



or



wherein (v) and (t) are independently 0 or a positive integer up to about 6;

(q) is zero or a positive integer;

L_1 and L_2 are independently selected bifunctional linkers;

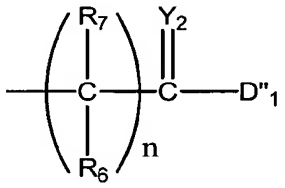
Y_{4-7} are independently selected from the group consisting of O, S and NR_{14};

R_{11-14} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} hetero-alkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroakoxy;

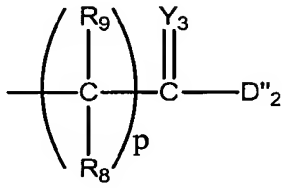
Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group;

B_1 and B_2 are independently selected from the group consisting of leaving groups, OH, residues of hydroxyl-containing moieties or amine-containing moieties;

E_{45} is



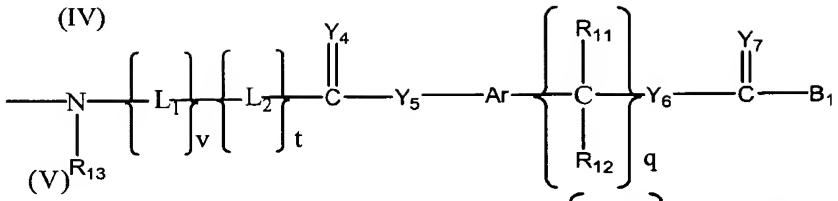
E_{46-48} are independently H, E_{45} or



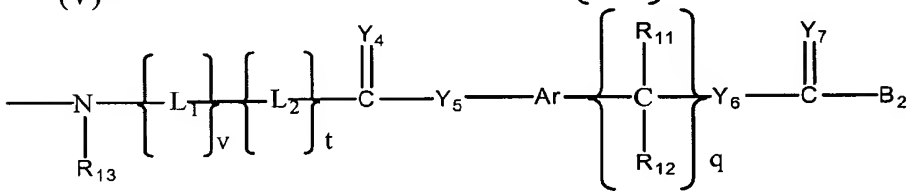
wherein

D''_1 and D''_2 are independently OH,

(IV)



or



5. The compound of claim 3, $Y_1$is O.

6. The compound of claim 1, wherein R_1 comprises a polyalkylene oxide residue.

7. The compound of claim 6, wherein R_1 comprises a polyethylene glycol residue.

8. The compound of claim 3, wherein R_1 comprises a polyethylene glycol residue.

9. The compound of claim 6, wherein R_1 is selected from the group consisting of $-C(=Y_8)-(CH_2)_f-O-(CH_2CH_2O)_x-A$, $-C(=Y_8)-Y_9-(CH_2)_f-O-(CH_2CH_2O)_x-A$, $-C(=Y_8)-NR_{20}-(CH_2)_f-O-(CH_2CH_2O)_x-A$, $-(CR_{21}R_{22})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-A$, $-NR_{20}-(CH_2)_f-O-(CH_2CH_2O)_x-A$, $-C(=Y_8)-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-C(=Y_8)-$, $-C(=Y_8)-Y_9-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-Y_9-C(=Y_8)-$, $-C(=Y_8)-NR_{20}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{20}-C(=Y_8)-$, $-(CR_{21}R_{22})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-O-(CR_{21}R_{22})_e-$, and $-NR_{20}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{20}-$

wherein:

Y_8 and Y_9 are independently O, S or NR_{20};

x is the degree of polymerization;

R_{20}, R_{21} and R_{22} are independently selected from among H, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

e and *f* are independently zero, one or two; and

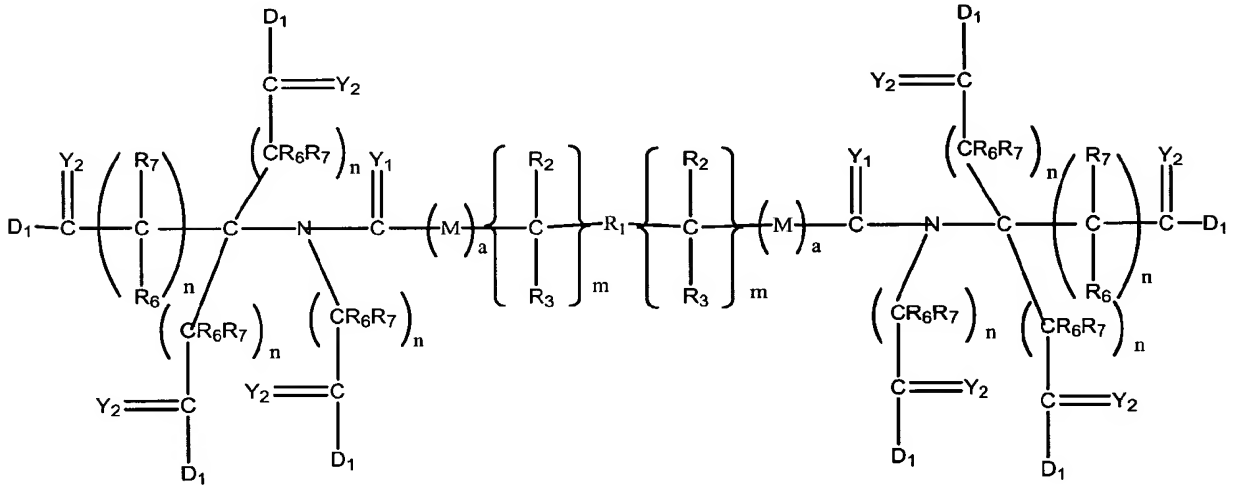
A is a capping group.

10. The compound of claim 9, wherein R_1 comprises -O-$(CH_2CH_2O)_x$ and x is a positive integer so that the weight average molecular weight is at least about 20,000.

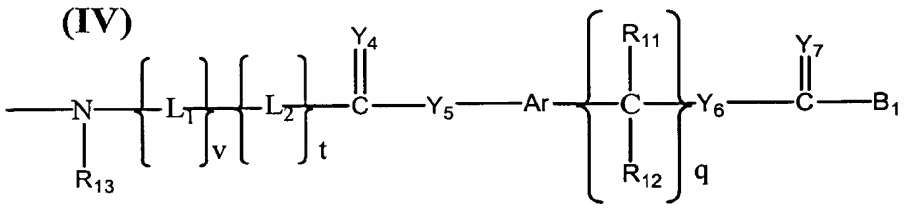
11. The compound of claim 3, wherein R_1 has a weight average molecular weight of from about 20,000 to about 100,000.

12. The compound of claim 3, wherein R_1 has a weight average molecular weight of from about 25,000 to about 60,000.

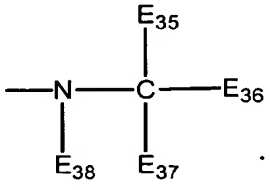
13. A compound of claim 3, comprising the formula



14. The compound of claim 13, wherein D_1 is



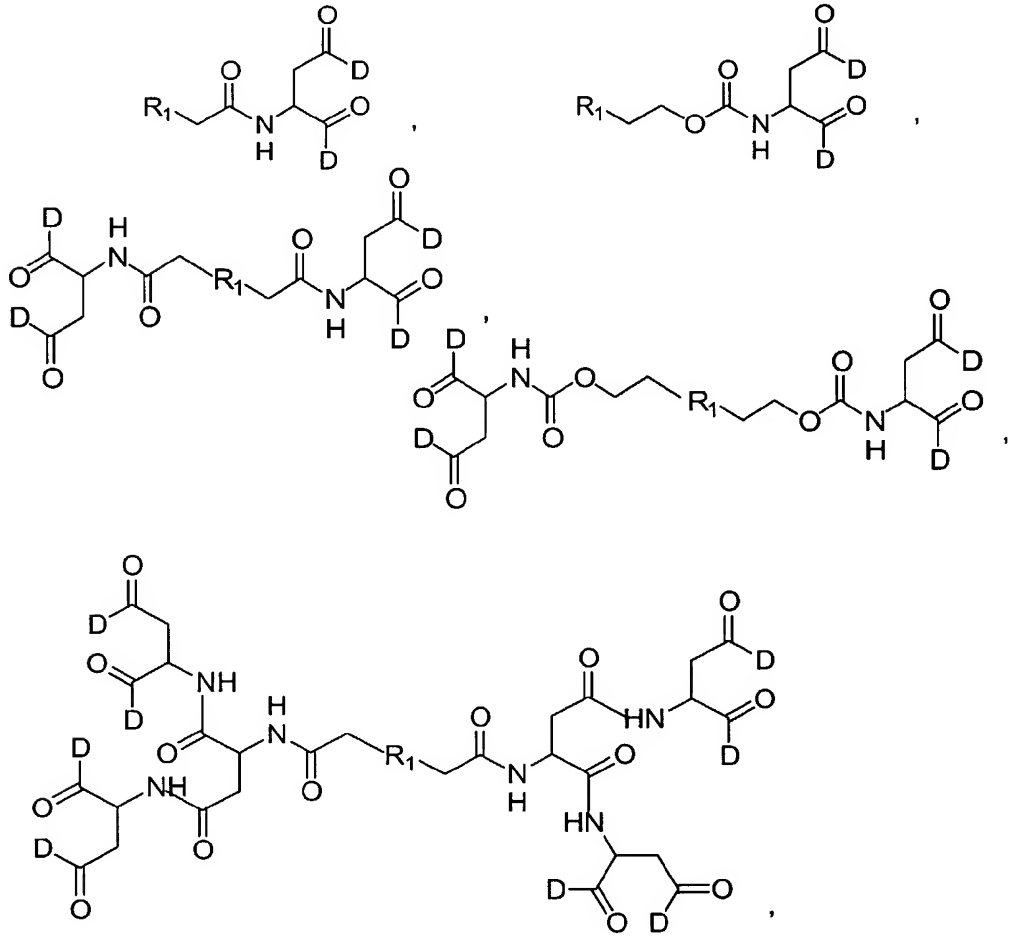
15. The compound of claim 13, wherein D_1 is

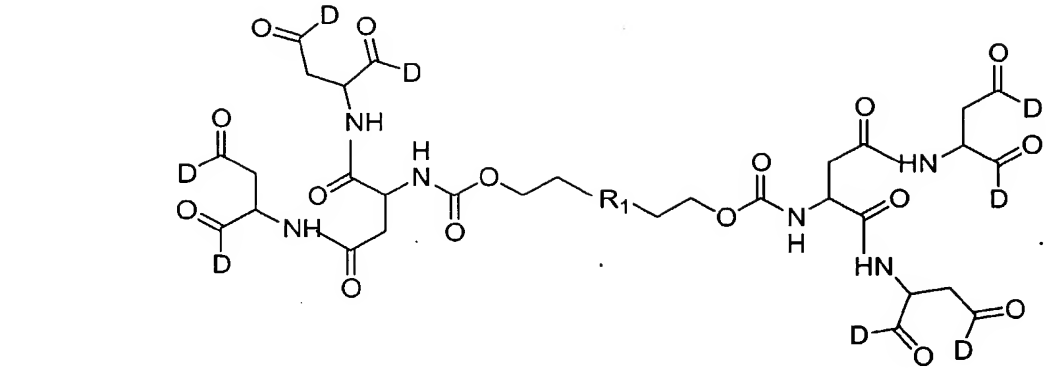


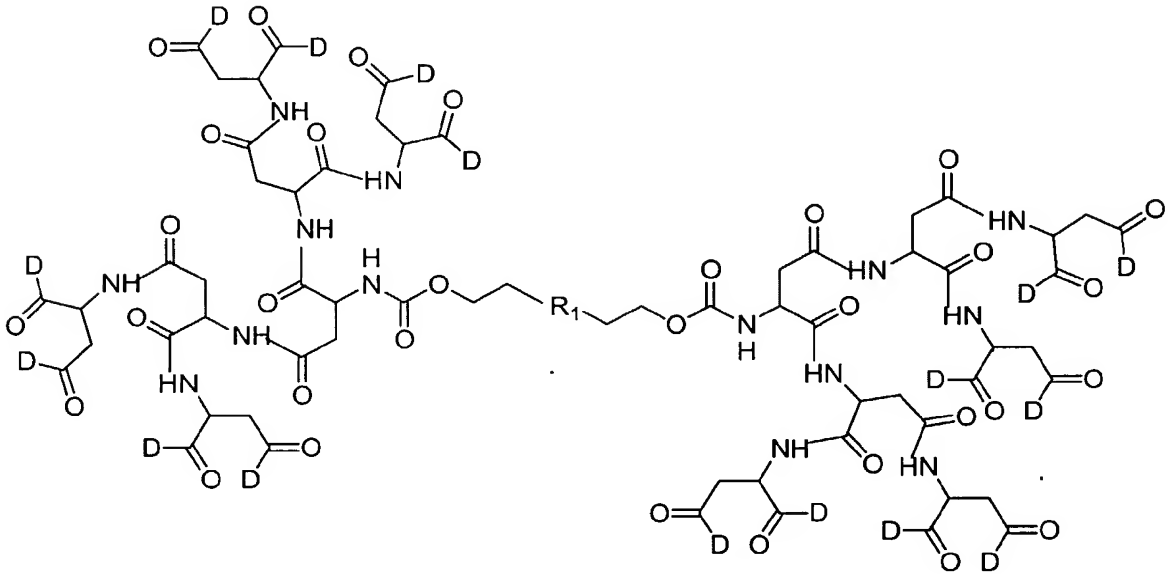
16. The compound of claim 1, wherein L_1 is $(CH_2CH_2O)_2$.

17. The compound of claim 1, wherein L_2 is selected from the group consisting of $-CH_2-$, $-CH(CH_3)-$, $-CH_2C(O)NHCH(CH_3)-$, $-(CH_2)_2-$, $-CH_2C(O)NHCH_2-$, $-(CH_2)_2-NH-$, $-(CH_2)_2-NH-C(O)(CH_2)_2NH-$ and $-CH_2C(O)NHCH(CH_2CH(CH_3)_2)-$.

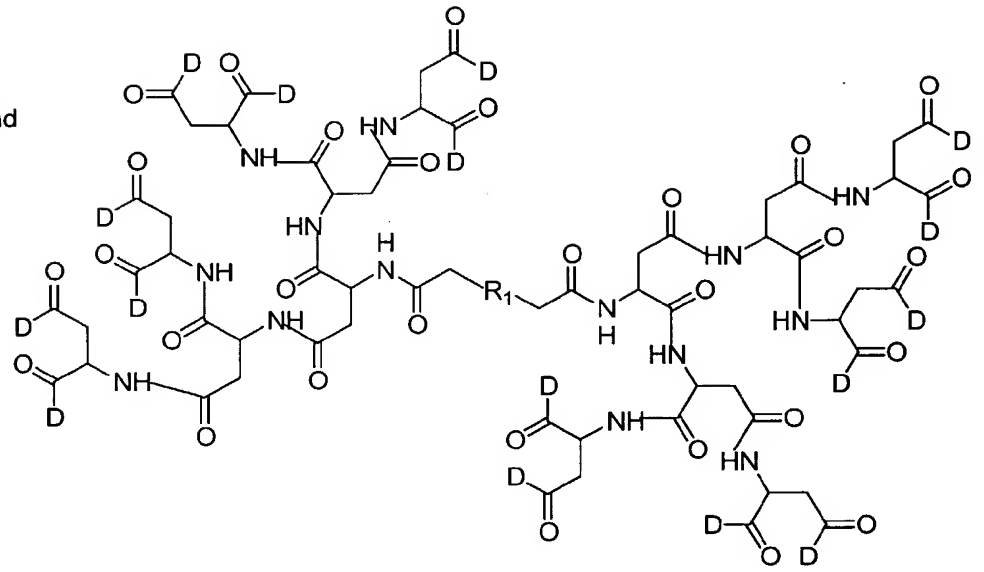
18. A compound of claim 1, selected from the group consisting of:



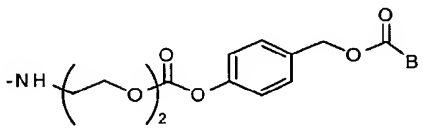


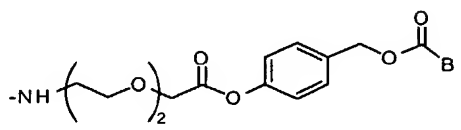


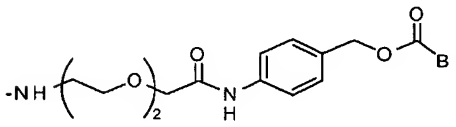
and

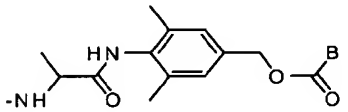


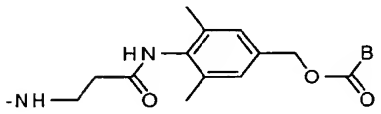
wherein R_1 is a PEG residue and D is selected from the group comprising:

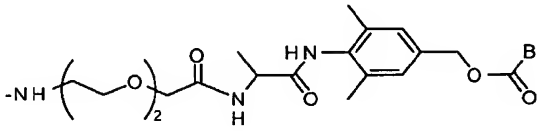


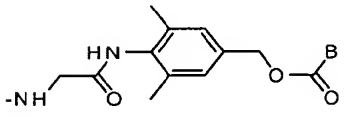


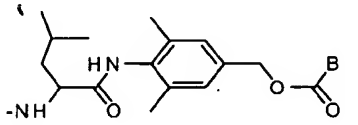


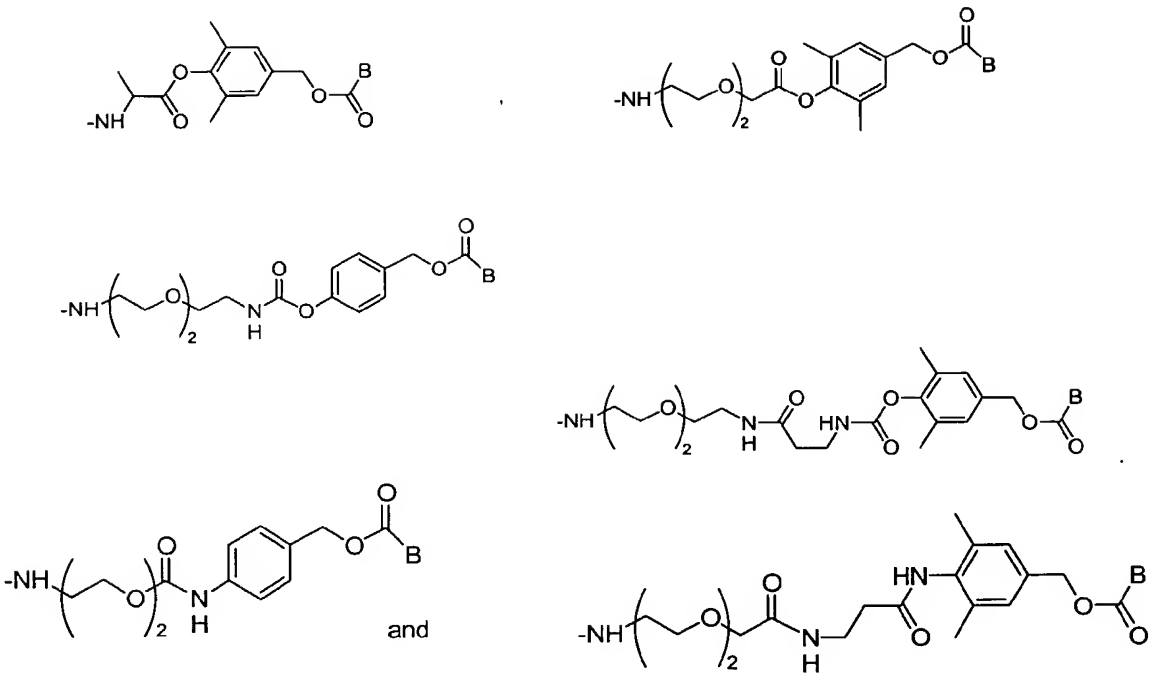












and

where B is a residue of an amine or a hydroxyl- containing drug.

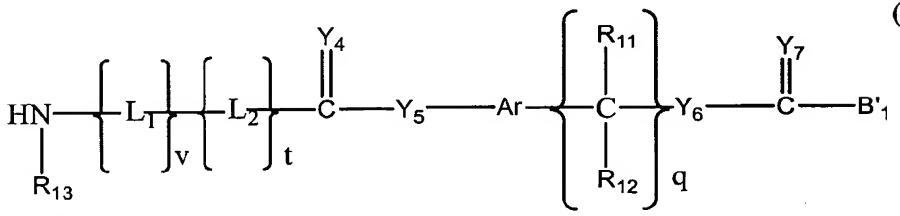
19. A compound of claim 18, wherein B is a residue of a member of the group consisting of: daunorubicin, doxorubicin; *p*-aminoaniline mustard, melphalan, Ara-C (cytosine arabinoside), leucine-Ara-C, and gemcitabine

20. A method of treatment, comprising administering to a mammal in need of such treatment an effective amount of a compound of claim 1, wherein D_1 is a residue of a biologically active moiety.

21. A method of treatment, comprising administering to a mammal in need of such treatment an effective amount of a compound of claim 18.

22. A method of preparing a polymer conjugate, comprising:

reacting a compound of the formula (VIII):



(VIII)

wherein (v) and (t) are independently 0 or a positive integer up to about 6;

L_1 and L_2 are independently selected bifunctional linkers;

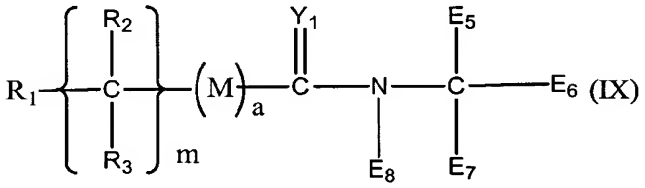
Y_{4-7} are independently selected from the group consisting of O, S and NR_{14};

R_{11-14} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group; and

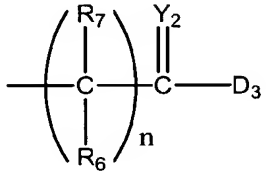
B'_1 is a residue of a hydroxyl- or an amine-containing moiety;

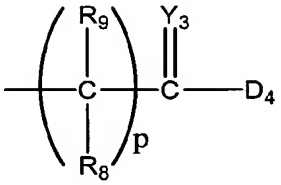
with a compound of the formula (IX):



(IX)

wherein

E_5 is 

E_{6-8} are independently H, E_5 or 

wherein

D_3 and D_4 are independently OH, a leaving group which is capable of reacting with an unprotected amine or hydroxyl or a terminal branching group;

R_1 is a polymeric residue;

Y_1 is O, S or NR_4;

M is O, S or NR_5;

(n) and (p) are independently 0 or a positive integer;

Y_{2-3} are independently O, S or NR_{10}; and

R_{2-10} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

under conditions sufficient to cause a polymeric conjugate to be formed.

Figure 1

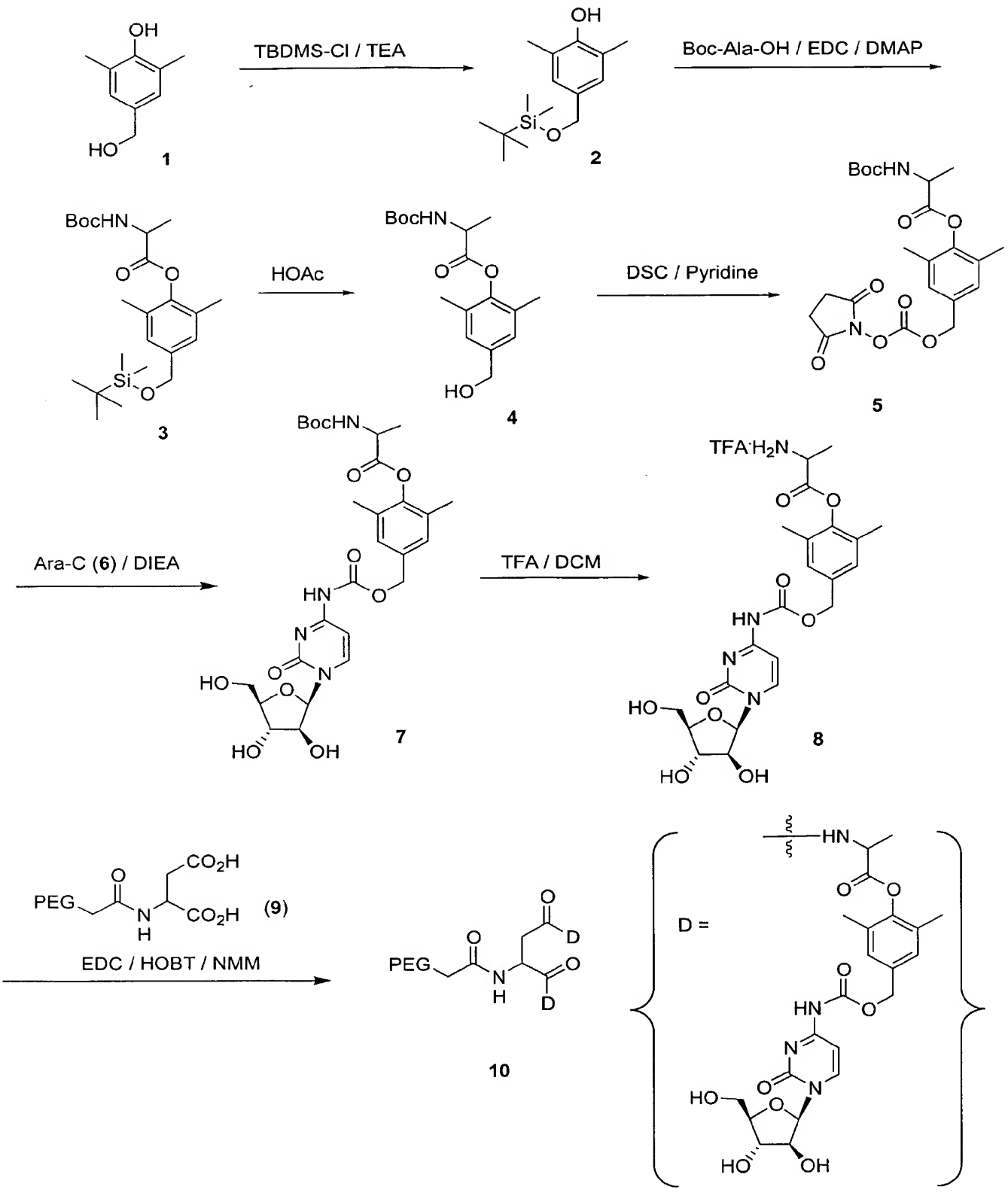

TBDMS-Cl / TEA
Boc-Ala-OH / EDC / DMAP
HOAc
DSC / Pyridine
Ara-C (6) / DIEA
TFA / DCM
EDC / HOBT / NMM
(9)
D =
1
2
3
4
5
7
8
10

Figure 2

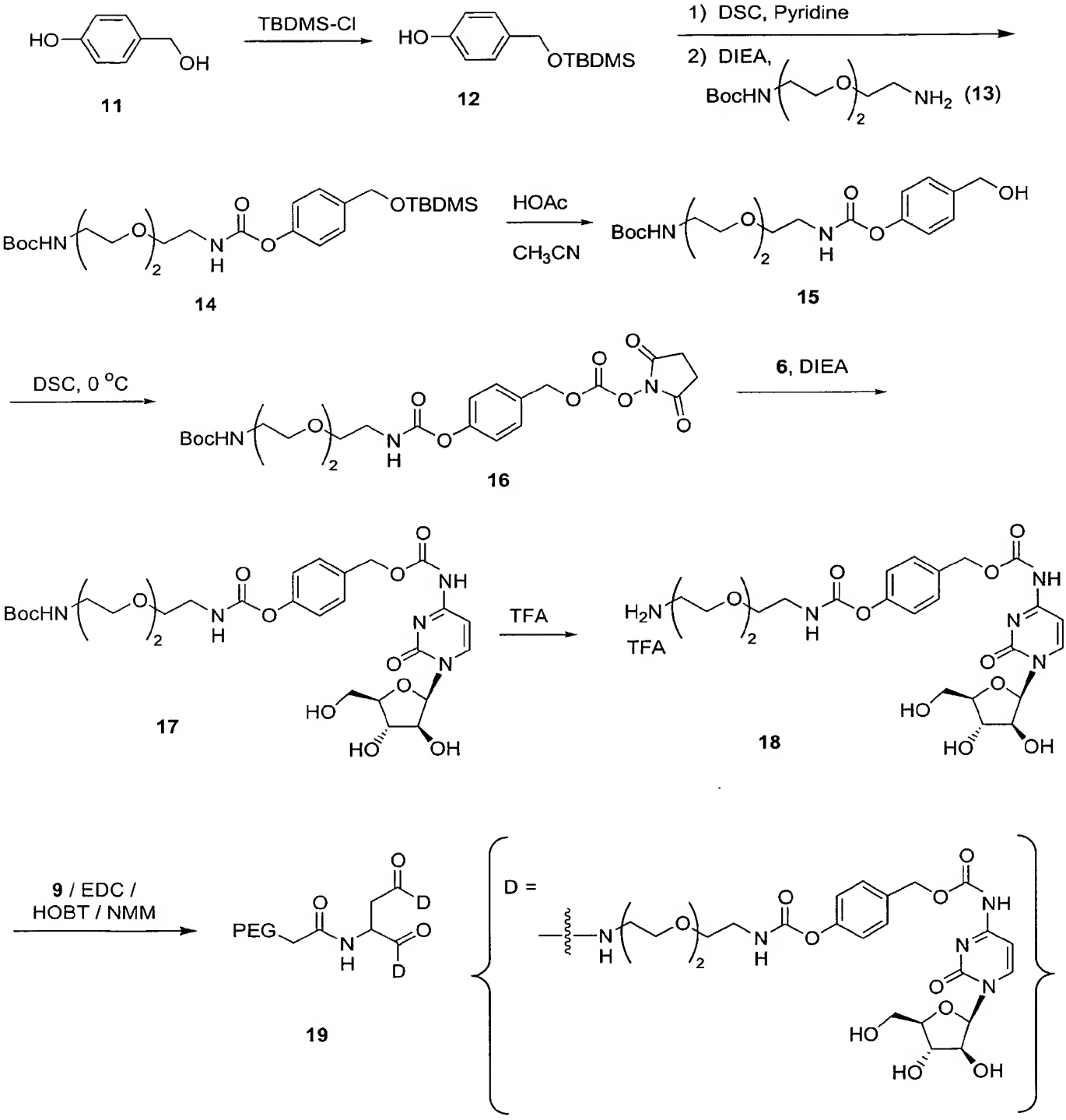

TBDMS-Cl
1) DSC, Pyridine
2) DIEA,
(13)
11
12
14
HOAc
CH_3CN
15
DSC, 0 °C
6, DIEA
16
17
TFA
18
9 / EDC /
HOBT / NMM
19
D =
PEG
BocHN
NH_2
TFA

Figure 3

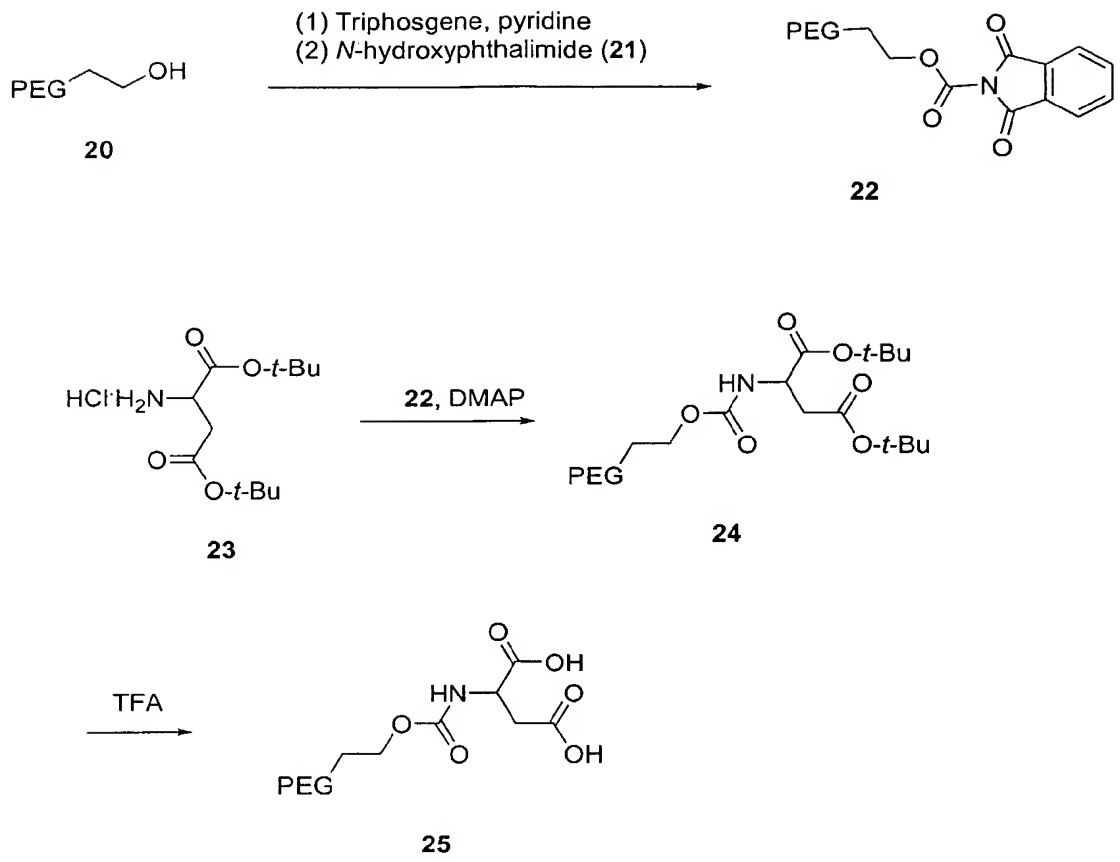

(1) Triphosgene, pyridine
(2) N-hydroxyphthalimide (21)
PEG
OH
20
22
O-t-Bu
HCl·H2N
23
22, DMAP
24
TFA
25

Figure 4

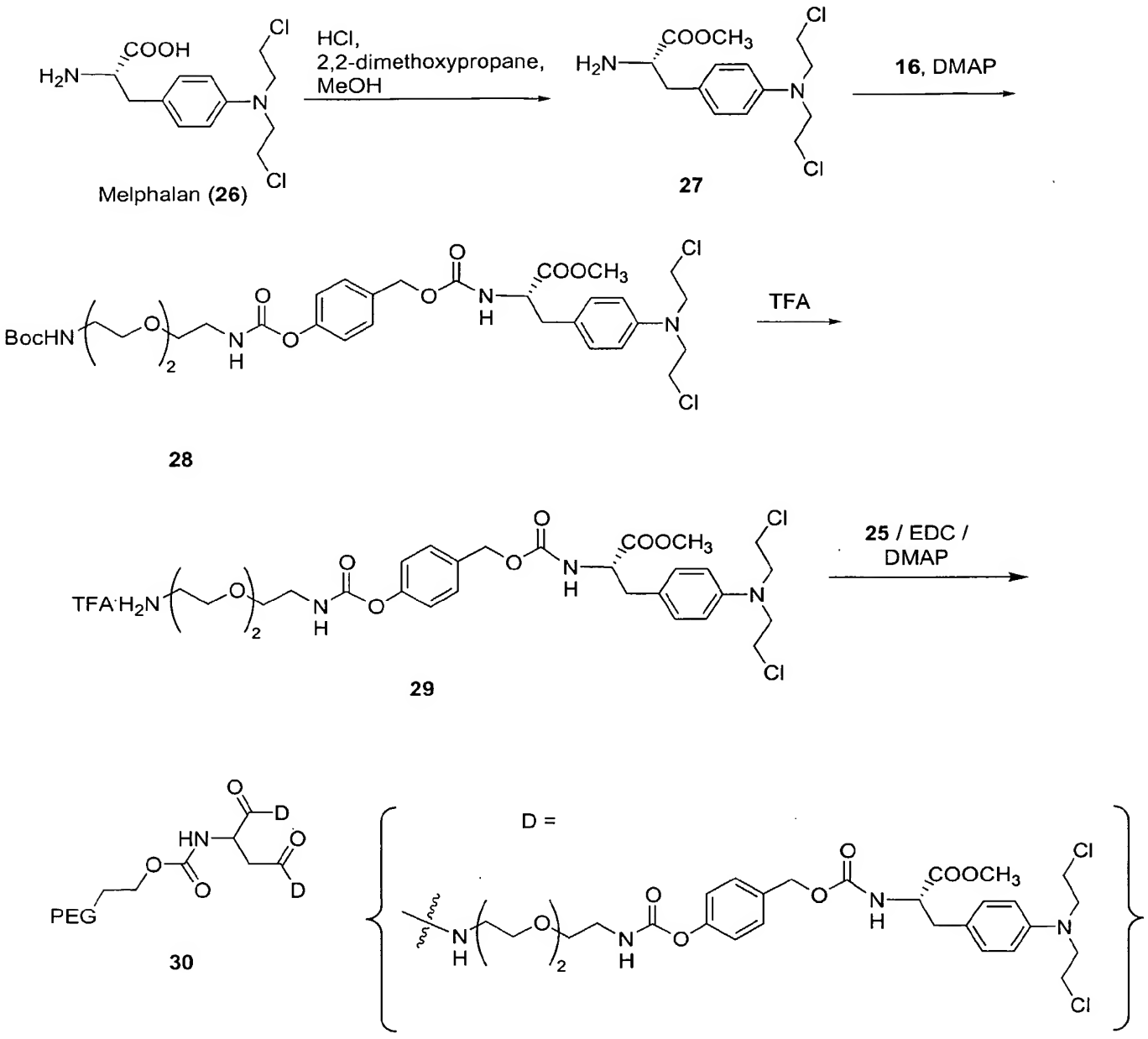
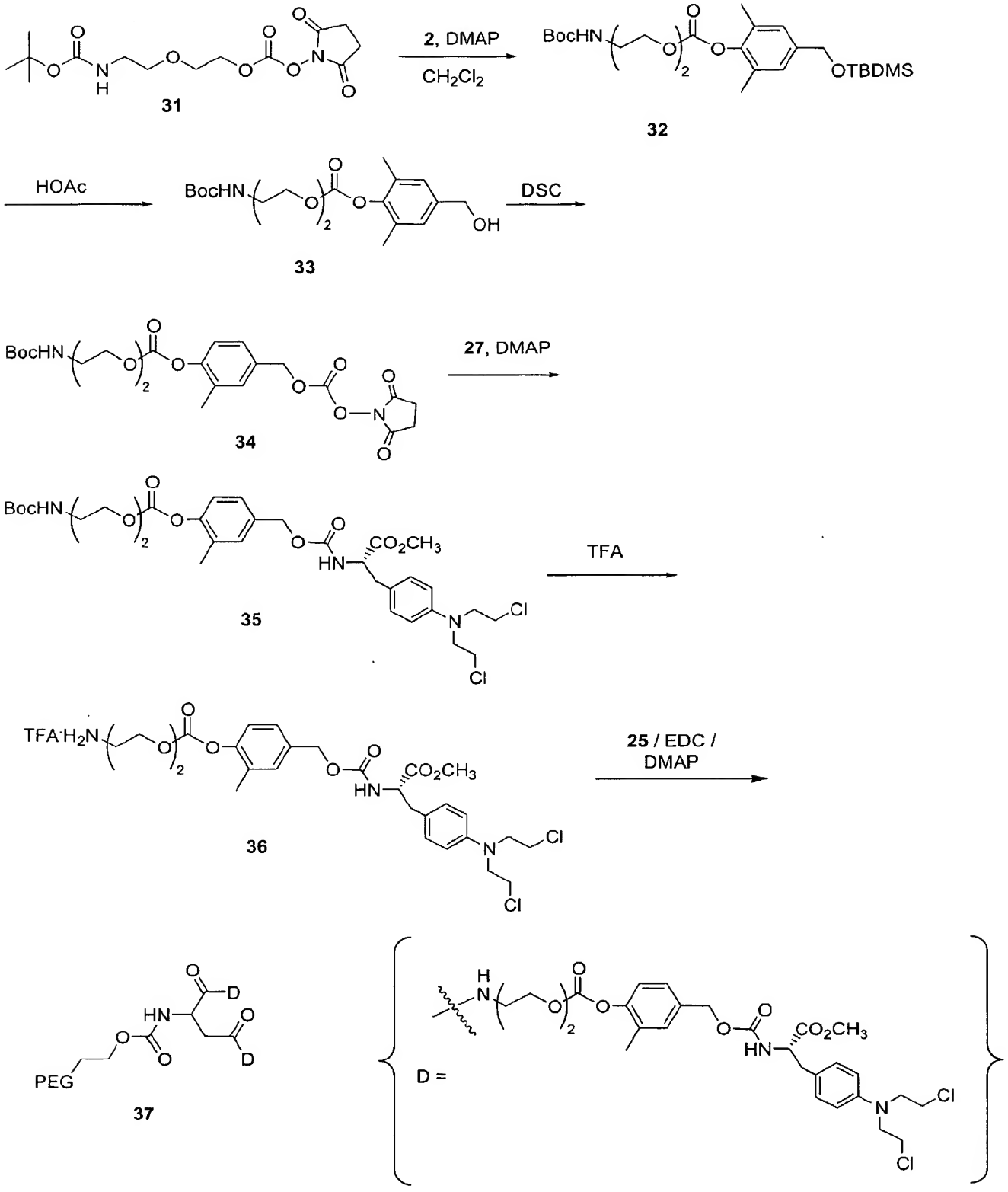

HCl,
2,2-dimethoxypropane,
MeOH
16, DMAP
Melphalan (26)
27
TFA
28
25 / EDC /
DMAP
29
30
D =
PEG

Figure 5


31
2, DMAP
CH2Cl2
BocHN
OTBDMS
32
HOAc
DSC
OH
33
27, DMAP
34
CO2CH3
Cl
35
TFA
TFA·H2N
36
25 / EDC / DMAP
PEG
37
D =